FACTORS AFFECTING THE UTILIZATION OF PROTEIN IN SOYBEAN MEAL BY DAIRY COWS

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CHAPTER I

INTRODUCTION

World demand for protein has increased the need to use feed protein efficiently. Our understanding of ruminant protein and amino acid metabolism has increased tremendously since Loosli et al. (1949) and Duncan et al. (1953) first reported that rumen microbes can synthesize all the essential amino acids from non-protein nitrogen (NPN). During the 1950s (Weller et al., 1958) and in the 1960s (Oltjen, 1969; Virtanen, 1969), dietary N source was generally regarded as irrelevant for ruminants, since microbial protein synthesis supplied sufficient quantities of amino acids for most levels of production. However, increasing the supply of protein or amino acids post-ruminally could increase N retention and animal performance. Chalmers et al. (1954), Egan (1965) and Little and Mitchell (1967) demonstrated that N retention was increased when casein was infused post-ruminally. Hence, ruminants will respond to an increased supply of post-ruminal protein. Schelling and Hatfield (1967) reported that N retention was increased when methionine-supplemented casein was infused abomasally into lambs. Tagari et al. (1962), Sherrod and Tillman (1964) and Glimp et al. (1967) successfully increased N retention or rate of gain by heating soybean meal to reduce its degradation in the rumen.

In response to these research results, one objective in protein nutrition of ruminant animals became to increase ruminal escape of high quality feed protein while maximizing bacterial protein supply to the small intestine (Owens, 1978). Understandably, the methods to achieve such goals became complex. Manipulating ruminant protein digestion can boost the efficiency of feed use by

ruminants and could increase the value both of inexpensive NPN sources, like urea, and of protein sources which escape ruminal digestion.

The protein requirements of high producing dairy cows are high. Cows must absorb large quantities of amino acids from the small intestine to meet the needs for milk protein synthesis, body tissue maintenance, gluconeogenesis, and fetal growth. During peak lactation, the quantity of amino acids absorbed from the small intestine may not be adequate. This restricts milk production and causes the cow to mobilize protein stores to meet the added need. Hence, much research has attempted to maximize the quantity and quality of protein reaching the small intestine for absorption to increase milk production (Netemeyer et al., 1982). For growing animals, as well, increased rates or efficiencies of growth have been observed when protected protein or protein slowly degraded in the rumen has been added to diet (Thomas et al., 1979; Stock et al., 1981). Besides increasing productivity, increased escape should reduce feed costs by increasing the potential for NPN use. Most bacterial nitrogen passes through the ammonia pool in the rumen (Pilgrim et al., 1970) so it is most efficient and cost effective to meet the nitrogen requirements of ruminal bacteria with NPN when a protected or slowly degraded protein is fed to increase the supply of high quality dietary protein to the small intestine. Supplementing a diet containing a high proportion of escape protein with NPN may be essential since, otherwise, ruminal bacteria can be starved for ammonia.

Infusion of casein or amino acids post-ruminally has increased milk production by 1 to 4 kg per cow per day (Clark, 1975a). Since post-ruminal infusion is not practical for application under field conditions, other methods to increase protein escape have been investigated. These include: 1) encapsulating amino acids with resistant coatings, 2) supplementing the diet with amino acid analogs, and 3) chemically or physically treating dietary proteins to reduce

ruminal degradation (Broderick, 1975; Clark, 1975b). The use of amino acid analogs and protected methionine in dairy cattle diets has been extensively investigated with emphasis on feeding the hydroxy analog of methionine (MHA). Dietary supplementation with MHA or ruminally protected methionine has produced little or no increase in milk yield but often has increased the percentage of total solids, fat and protein in milk (Clark, 1975b; Yang et al., 1984).

To increase ruminal escape of dietary protein, one can formulate ruminant diets using feed ingredients low in protein solubility (Wohlt et al., 1976; Braund et al., 1978; Davis, 1978; Majdoub et al., 1978). Feeds low in protein solubility tend to be degraded less extensively in the rumen (Satter et al., 1977). Though the relationship is far from perfect, milk production has been increased when cows were fed diets low in protein solubility in some studies although results have not all been positive (Davis, 1978; Ahrar and Schingoethe, 1979; Netemeyer et al., 1982). In these studies, milk production may have been increased either due to a reduction in the soluble nitrogen content of the diets or because of alteration in other dietary factors such as levels of non protein nitrogen or soluble carbohydrates and levels or patterns of dietary or bypassed amino acids.

The objective of this dissertation was to evaluate the impact of soybean meal protein solubility on milk production, milk composition, and ruminal degradation. This thesis work was designed to determine the extent of ruminal proteolysis, ruminal fractional passage rate and production response to several modified soybean meals incorporated into diets for lactating dairy cows.

CHAPTER II

REVIEW OF LITERATURE

Soybean meal protein degradation and utilization by ruminants has been an active area of research. Investigations with ruminants have employed in vitro, in situ and in vivo measurements. This review will discuss nitrogen metabolism and the potential for protecting dietary protein from microbial attack in the rumen. Next, methods to alter the site of protein degradation will be discussed and finally recent trials evaluating protein solubility of diets for lactating dairy cattle will be reviewed.

Nitrogen Metabolism in the Rumen

The nitrogen in most feedstuffs consists largely of protein-N with small amounts of NPN. Nitrogen enters the rumen in feed and saliva and by transfer across the rumen wall (Hogan, 1975). Plant proteins vary greatly in structure, amino acid composition, solubility and susceptibility to ruminal degradation. Degradation of protein in the rumen by the bacterial and protozoal populations can be extensive. Proteins are hydrolyzed by proteolytic bacteria and constituent amino acids are deaminated to ammonia, short chain fatty acids, and carbon dioxide (Tillman and Sidhu, 1969; Allison, 1970). Many factors including protein solubility, method of processing, ruminal pH, rate of passage, and feed intake level can influence either the rate or extent of degradation of dietary plant protein in the rumen (Clark, 1975b; Satter et al., 1977).

Sources of dietary NPN include all nitrogenous compounds which are not proteins such as amino acids, peptides, amides, amines, ammonium salts, nitrates,

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nitrites and urea (Chalupa, 1978). Apart from the NPN derived from natural feeds, ammoniated feeds or other nitrogenous compounds often are added to the diet. The value of NPN to the ruminant depends upon the extent to which ammonia liberated from NPN is utilized by rumen bacteria for protein synthesis (Chalupa, 1972). The rate and amount of ammonia released from NPN compounds depends on the presence and activity of enzyme systems which degrade NPN.

The work of Zuntz and Hagemann in 1891 (see Hungate, 1966) first demonstrated the usefulness of NPN for ruminant animals. Subsequently, many workers studied the ruminal metabolism of NPN compounds, particularly urea. Urea is readily hydrolyzed to ammonia in the rumen (Pearson and Smith, 1943; Jones, 1967) and ammonia is the predominant nitrogen source used by rumen bacteria for protein synthesis (Bryant and Robinson, 1962). Therefore, dietary urea N or urea recycled to the rumen directly from the bloodstream (Houpt, 1970) or via saliva (Bartley, 1976) contributes ammonia-N for the synthesis of microbial protein.

With animals fed typical diets, an average of 10% of total nitrogen supply reaches the rumen via salivary nitrogen recycling (Bartley, 1976). He estimated that a 700 kg cow consuming a hay and grain diet recycles 30 to 80 g of nitrogen each day. Urea nitrogen totals some 75 to 85 % of the salivary nitrogen of cattle (Phillipson and Mangan, 1959; Bailey and Balch, 1961). Experiments with sheep have demonstrated that salivary urea nitrogen concentration is correlated with rumen ammonia concentration (Lewis, 1957), blood urea concentration, nitrogen intake (Somers, 1961b), and saliva secretion rate (Somers, 1961a), whereas total saliva production is related to the amount and type of feed consumed. Only 15% of total urea entering the rumen was salivary urea; this indicates that the entry of urea from the blood is the more important.

Jones et al. (1964) found that 35% of the bacteria isolated from the rumen of sheep fed urea, barley, and hay produced urease. No urea-producing bacteria were isolated and protozoa appeared to have little, if any, urease activity. More recently, John et al. (1974) isolated an ureolytic strain D of Selenomonas ruminantium from rumen fluid. Urease production by these bacteria increased when the medium contained lower concentrations of ammonia-N; however, urease production was repressed when large amounts of urea were included in the growth medium. Houpt (1970) observed greater transfer of urea into Pavlov pouches contaminated with rumen microorganisms than into rinsed, sterile pounches, and suggested that urease within the rumen epithelium may aid in transfer of urea from the blood to the rumen.

Canadian workers (Cheng and Costerton, 1980) introduced the concept of considering rumen microorganisms as existing in three phases: the fluid phase, attached to feed particles and adherent to the rumen epithelium. Investigators in rumen microbiology have isolated a distinct flora inhabiting the rumen epithelium (Bauchop et al., 1975). This facultatively anaerobic flora appears to have become specialized in digesting exfoliatory epithelial tissue and hydrolyzing urea and appears to be independent of the substrate fermenting in the rumen. This bacterial population may also assist in controlling recycling of urea via the blood. Cheng and Wallace (1979) demonstrated that urease activity was greatest when the ammonia concentration was low and decreased as rumen ammonia concentration increased. The urease activity may assist in increasing recycling of urea during periods of low dietary protein intake. The faster urea is hydrolyzed to ammonia, the greater the concentration gradient across the rumen wall. More urea from the blood can be recycled under conditions where N is limited for the rumen microorganisms.

Microbial Metabolism in the Rumen

Proteins entering the rumen are catabolized to peptides, amino acids, and ammonia at rates largely dependent on protein solubility (Hendrickx and Martin, 1963; Smith, 1975). Protein digestion occurs by cell-bound bacterial proteases and following protozoal engulfment of particles (Sutherland, 1976). Proteolytic ruminal bacterial include species of Bacteroides, Selenomonas and Butyrivibrio although no single species is particularly active (Bryant, 1977; Chalupa, 1978). Ruminal proteases degrade proteins to peptides and amino acids to ammonia. Cell growth, and thus the synthesis of bacterial protein, is usually limited by the availability of energy for rumen bacteria. Consequently, substrates must be in close proximity to bacterial cells for hydrolysis and rates of proteolysis are positively related to bacterial cell numbers (Hogan and Hemsley, 1976). Having proteases cell bound aids uptake of products of proteolysis for cell growth. Though the protease of Bacteroides amylophilus is cell-bound (Blackburn, 1968), this species does not use end products of proteolysis. Pure cultures use ammonia as the predominant nitrogen source for synthesis of protein (Bryant and Robinson, 1962; Abou-Akkada and Blackburn, 1963). In the rumen, some species of bacteria use small quantities of peptides and amino acids that are available, but the majority can survive with ammonia from catabolized peptides and amino acids as their sole source nitrogen source (Allison, 1970; Bryant, 1977).

Proteolytic protozoa include species of Entodinium, Isotrichia, Eudiplodinium, and Ophryoscolex (Allison, 1970). Engulfed bacterial cells are the major source of nitrogen for protozoa. Bacterial proteins are hydrolyzed to free amino acids which are incorporated into protozoal protein (Coleman, 1967; Wallis and Coleman, 1967). The relative contribution of protozoa and bacteria to ruminal proteolysis is directly related to their biomass in the rumen. Factors affecting the rate of proteolysis include ruminal pH and temperature, time

available for reaction, concentration of enzymes and substrates, and the presence of inhibitors (Hogan and Hemsley, 1976).

Controversy exists whether deamination or proteolysis is the rate-limiting step in protein degradation. Early investigations by Annison et al. (1959) and Lewis (1962) suggested that amino acids rarely accumulate in the rumen, even following feeding large amounts of digestible protein. In contrast, Leibholtz (1965), Demeijer and Van Nevel (1967) and Hendrickx et al. (1972) reported that free amino acids can be detected shortly after feeding and Russell and Martin (1984) recently suggested that peptides are extensively used by ruminal bacteria.

Nitrogen Products Leaving the Rumen

Protein entering the rumen may be metabolized by microorganisms or passed to the lower digestive tract unaltered. The three predominant nitrogen-containing fractions leaving the rumen are microbial protein, undegraded feed protein and endogenous protein. A number of techniques have been used to differentiate between microbial and undegraded feed protein (Smith, 1975). Estimates of microbial protein vary by 10 to 36% depending on the microbial marker chosen (Theurer, 1979).

Fractionation procedures to distinguish between microbial and feed protein generally rely on specific compounds or markers found only in microbes. Common microbial markers include: nucleic acids (McAllan and Smith, 1969), diaminopimelic acid (Czerkawski, 1974), aminoethylphosphoric acid (Czerkawski, 1974), ^{32}P (Smith et al. 1978), ^{35}S (Kennedy et al., 1980), total purines (Zinn and Owens, 1982) and D-alanine (Garrett et al. 1982).

From numerous studies where these different methods were employed to assess the proportion of microbial nitrogen reaching the proximal duodenum of ruminants fed a variety of diets, it was concluded that 40 to 80% of total nitrogen

reaching the lower gut is of microbial origin (Smith, 1975). Several reviews (Smith, 1975; Czerkawski, 1978; Stern and Hoover, 1979; Bergen et al., 1982; Schelling et al., 1982; Theurer, 1982) have compared the estimates of microbial protein based on a number of microbial markers. Though variability is high, no single marker appears more useful than the others and each has advantages and disadvantages.

By difference between duodenal non-ammonia-N flow and microbial N, flow of feed protein from the rumen can be calculated. Extent of degradation of protein in the rumen is dependent on several factors, the most important of which are protein solubility and retention time (Satter et al., 1977). The amount of most feed proteins degraded in the rumen ranges from 30 to 80% with 60% being a reasonable average (Chalupa, 1975; Satter and Roffler, 1975; Sutherland, 1976) as shown in Table 1. From the metabolizable protein scheme of Satter and Roffler (1975) an estimate of 62% can be derived.

Numerous research studies have been conducted to determine the effect of diet on the relative proportion of nitrogen that leaves the rumen as microbial nitrogen, as undegraded plant protein, and by diffusion across the rumen wall. The challenge has been to minimize degradation of protein in the rumen without decreasing microbial protein synthesis and thereby to increase the total amount of dietary protein reaching the lower gut (Chalmers et al., 1954; Tagari et al., 1962; Reis and Tunks, 1969).

Several techniques can be used to minimize degradation of nitrogenous compounds in the rumen and to increase rumen bypass of amino acids or protein. These include selection of high escape ingredients, physical processing, heat or chemical treatment, and post-ruminal infusion of protein (Bull, 1981). If animal performance increases when these techniques are used, the response can be attributed to one or more of the following: 1) an increased quantity of total

Table 1. TENTATIVE ESTIMATES OF UNDEGRADED PROTEIN FOR COMMON FEEDSTUFFS WHEN TOTAL DRY MATTER INTAKE IS IN EXCESS OF 2 PERCENT OF BODY WEIGHT[a]

Feed	Number of Measurements	Mean Fraction of Undegraded Protein	Standard Deviation
Feed grains			
Barley	2	0.21	0.07
Corn	3	0.65	0.06
Sorghum grain	8	0.52	0.15
Oil meals			
Cottonseed meal (solvent)	6	0.41	0.12
Cottonseed meal (prepress)	2	0.36	0.02
Cottonseed meal (screw press)	2	0.50	0.07
Linseed meal	1	0.44	--
Peanut meal	2	0.30	0.08
Rapeseed meal	1	0.23	--
Soybean meal	10	0.28	0.14
Sunflower meal	2	0.24	0.05
By-product feeds			
Blood meal	1	0.82	--
Brewers dried grains	5	0.53	0.14
Corn gluten meal	3	0.55	0.06
Distillers dried grains	2	0.62	0.07
Fish meal	4	0.80	0.12
Meat meal	1	0.76	--
Meat and bone meal	2	0.60	0.11
Forages			
Alfalfa hay	4	0.28	0.08
Alfalfa (dehydrated)	3	0.62	0.04
Bromegrass hay	2	0.32	0.12
Corn silage	1	0.27	--
Timothy hay	2	0.42	0.11

[a]National Research Council. 1985. Ruminant Nitrogen Usage. National Academy of Sciences National Research Council. Washington, D.C.

amino acids reaching the duodenum (Sharma et al., 1974), 2) reduced nitrogen loss due to decreased urinary excretion of urea (Reis and Tunks, 1969), 3) greater quantities of limiting amino acids available for absorption in the small intestine (Clark, 1975a), 4) an increased supply of carbon for gluconeogenesis (Clark, 1975a), 5) higher quality proteins reaching the duodenum (Little and Mitchell, 1967), or 6) changes in hormonal status (Clark, 1975a; Ferguson, 1975).

Protein Degradation Rate

In 1972, Mangan infused casein and casein hydrolysate in vivo and demonstrated that deamination was more rate-limiting than proteolysis in protein degradation of a soluble protein. Whether deamination or proteolysis is rate-limiting in protein degradation depends upon independent factors affecting rumen nitrogen metabolism, such as rate of degradation of dietary protein, rumen pH, or dietary energy level.

The extent of nitrogen degradation occuring in the rumen is a function of rate of protein degradation and retention time (rate of passage of protein out of the rumen). Pichard and Van Soest (1977) established a model for protein degradation which separated protein into three fractions based on rate of breakdown. The A fraction contains rapdily solubilized non-protein nitrogen; the B fraction is subdivided into two or more categories: rapidly degraded and more slowly degraded protein; and the C fraction is undegraded indigestible protein.

Protein solubility in the rumen was considered to be the most important factor affecting rate of protein degradation by these and certain other workers (Hendrickx and Martin, 1963; Sniffen, 1974). Solubilities of protein sources are partially determined by the specific classes of protein present. Feeds with the majority of total protein being albumin and globulin are more soluble whereas

feeds with greater amounts of glutelin and prolamine are less soluble (Wohlt et al., 1976).

Essentially all soluble nitrogen and only soluble protein was initially believed to be degraded in the rumen (Mertens, 1977). Current evidence indicates that nitrogen solubility under-predicts the extent of nitrogen degradation in the rumen though these measurements are usually correlated (Wohlt, 1973; Sniffin, 1974; Zinn et al., 1981). Generally speaking, the greater the solubility in rumen fluid, the more readily a feed component is degraded in the rumen. However, certain soluble proteins may resist attack. Mangan (1972) reported that ovalbumin, though highly soluble, is slowly degraded in the rumen. Ovalbumin is resistant to exopeptidase attack due to the cyclical arrangement of its polypeptide chain so ovalbumin lacks carbon or nitrogen terminal amino acid residues.

Mahadevan et al. (1980) reported that soluble and insoluble proteins of soybean meal (SBM) were hydrolyzed at equal rates by a protease isolated from Bacteroides amylophilus. These researchers also reported that soluble proteins of soybean meal, rapeseed meal, and casein were hydrolyzed at different rates by the protease though differences in extent of digestion were very small. It has been reported by Van der Aar et al. (1982) that soluble proteins of soybean meal treated with alcohols had lower rates of in vitro degradation as compared with soluble proteins of control soybean meal. In contrast to the report of Mahadevan et al. (1980), in vitro degradation rates of soluble and insoluble proteins of control soybean meal were not equal in other studies. Bull et al. (1977) summarized the relationship between solubility and degradability by stating that, while the ability of a protein to be solubilized in the rumen is related to rumen degradation, solubility does not ensure degradation, nor does insolubility infer that a protein is not degraded.

Protein structure also plays a role in ruminal protein degradation. Disulfide bridges, crosslinking, and helical structures appear to decrease protein degradation (Nugent and Mangan, 1978; Mahadevan et al., 1980). Altering the protein structure by alcohol treatment (Van der Aar et al., 1982), altering the protein structure by heating (Tagari et al., 1962; Sherrod and Tillman 1964; Glimp et al., 1967), or complexing amino groups with aldehydes (Hatfield, 1973) reduced protein degradation in the rumen.

Rumen pH alters both protein solubility and microbial proteolysis. Clark (1975a) stated that proteins were least soluble at their isoelectric point due to lack of a net charge and electrostatic repulsion between protein molecules. Fontaine and Burnette (1944) and Wohlt et al. (1973) reported that a low pH reduced protein solubility of many common protein supplements. Blackburn and Hobson (1960), and Lewis and Emery (1962) reported that the optimum pH for bacterial proteolysis and deamination was between 6 and 7. Russell et al. (1979) studied the effect of pH on the growth rate of five pure cultures of rumen bacteria. The ranking of the species with respect to growth rate was pH dependent, suggesting that pH may influence competition among bacterial species in the rumen.

Neudoerffer et al. (1971) compared ruminal protein degradation by cows fed high corn diets with that by cows fed high forage diets. They found protein degradation to be lower for the high corn treatment, but it was impossible to determine whether the response was due to low rumen pH on the high corn diet or the inherently lower degradation of corn protein.

The majority of the work conducted concerning the influence of pH on protein degradation has used <u>in situ</u> and <u>in vitro</u> techniques. Okeke et al. (1983) using <u>in situ</u> techniques, suspended nylon bags containing soybean meal in the rumen of steers fed concentrate diets supplemented with various levels of buffers

to alter ruminal pH. They concluded that there was a strong correlation between ruminal pH 4 h post-feeding and nitrogen disappearance from soybean meal suspended in the rumen for 24 h. In the in situ work by Loerch et al. (1983), cows were fed NaOH treated corn or high moisture corn. Sodium hydroxide treated corn maintained a higher ruminal pH while ruminal pH decreased with high moisture corn in the diet. Protein degradation of various protein sources was measured using in situ techniques. Corn treatment and level had an affect on rates of N disappearance. Disappearance rates from soybean meal and dehydrated alfalfa meal were affected more by pH than rates from blood meal, corn gluten meal or bone meal Because both in vitro and in situ solubility and degradation of soybean meal were reduced at a low pH, whereas in vitro solubility and in situ degradation of blood meal and corn gluten meal were low and relatively unchanged by pH, these workers concluded that protein solubility had more impact on ruminal degradation than did alteration in the bacterial population or proteolytic activity.

The second main factor that alters extent of ruminal protein degradation is rate of N passage out of the rumen. This factor is most important with proteins whose retention time permits microbial fermentation to be prolonged. Reducing food particle size, increasing intake level or frequency of feeding, and rate of fermentation were reported by Balch and Campling (1965) to decrease ruminal retention time. Increasing feed intake increases flow of undegraded protein out of the rumen (Mertens, 1977; Tamminga, 1979; Zinn and Owens, 1983). Increased protein escape was reportedly due to a reduced ruminal retention time. However, rumen pH was not reported in these studies and it is possible that, with higher feed intakes, rumen pH also was depressed. Reduced pH should reduce ruminal protein degradation. Feeding or infusing buffers such as sodium bicarbonate or mineral salts has been shown to increase rumen fluid dilution rate (Thomson et al., 1975; Rodgers et al., 1979). This could increase flow of undegraded protein out of

the rumen provided other factors such as increased pH do not alter rate of protein degradation. Increased passage rate increases efficiency of microbial growth both *in vitro* and *in vivo*. This is well documented in reviews by Hespell and Bryant (1979) and Harrison and McAllan (1980). However, the effect of passage rate on proteolysis in the rumen or flow of bacterial N out of the rumen has not been measured directly.

Methods for Measuring Protein Degradation and Solubility

Several rapid, inexpensive methods have been developed to indirectly estimate the extent of ruminal protein degradation. These techniques (Buttery and Cole, 1977; Oldham, 1977) avoid the problems associated with measuring degradation directly by use of microbial markers and animals prepared with abomasal or duodenal cannalae, but most of these procedures remain to be tested against *in vivo* escape values.

Solubility of protein by extraction in some solvents is one common procedure for evaluation of feedstuffs since generally solubility is positively correlated with ruminal protein degradation. Although autoclaved rumen fluid has been proposed as the standard to which other solvents have been compared, recent research (Wohlt et al., 1973; Crooker et al., 1978) has attempted to identify a solvent that extracts similar quantities of soluble protein, is more uniform in composition, and can be obtained more easily. Wohlt et al. (1973) compared Burroughs mineral buffer diluted to 10% with distilled water to autoclaved rumen fluid and found similar quantities of nitrogen were extracted from purified proteins by both solvents. Because this mineral buffer is difficult to prepare, has a short shelf life, and has a high background level of nitrogen, Crooker et al., (1978) investigated alternative solvents. In addition to autoclaved rumen fluid and Burroughs mineral

buffer, 0.15 M NaCl, a modified Burroughs solution which substituted Na_2SO_4 for $(NH_4)_2SO_4$ on an equimolar basis, and McDougal's artificial saliva were examined. No significant difference was found among the mean quantities of nitrogen extracted by either the Burroughs mineral buffer, autoclaved rumen fluid or sodium chloride. There was a high correlation (r = .93) between the amount of protein extracted by sodium chloride and that extracted by the Burroughs solvent. Dilute NaOH (0.02 N) also has been used as a solvent (Little et al., 1963; Craig and Broderick, 1978) but extracts greater quantities of soluble protein than other solvents. Craig and Broderick (1978) demonstrated a high correlation (r = 0.97) between protein solubility in NaOH and in vitro cottonseed meal protein degradation estimated by kinetic studies. Other solvents that have been used include water (Little et al., 1963; Goering and Waldo, 1974), 0.2 M phosphate buffer (MacRae, 1976), 0.1 N HCl solution (Brady, 1960), ethanol (Brady, 1960) and borate-phosphate buffer (Krishnamoorthy et al. 1982).

The quantity of protein extracted from feeds depends on the degree of agitation and length of extraction time as well as solvent temperature, pH, chemical composition, and ionic strength. Solubility of feedstuff protein in one solvent may be unrelated to solubility in another solvent (Little et al., 1963; Crooker et al., 1978; Waldo et al., 1979) possibly because of varying inter- and intramolecular forces acting between the protein and ions of each solvent (Crooker et al., 1978).

Another procedure used to predict in vivo protein degradation is to measure ammonia accumulation after incubation in vitro with rumen fluid. This technique often underestimates in vivo degradation because of ammonia uptake to support microbial growth. This is especially apparent with rapidly fermented feeds in which ammonia concentrations in vitro decrease rather than increase (Annison et al., 1954). Failure of this method to consider ruminal retention time limits its

usefulness to predict in vivo degradation.

Disappearance of protein from dacron bags suspended in the rumen has been used to estimate rumen degradation of feed proteins (Mathers et al., 1977; Mehrez and Ørskov, 1977; Mohamed and Smith 1977; Ørskov and Mehrez 1977; Crawford et al., 1978; Stern et al., 1978; Ørskov and McDonald, 1979). Maximum protein degradation in the rumen has been assumed to be that reached when 90% of the digestible dry matter of the feed has disappeared from the dacron bag (Ørskov and Mehrez, 1977).

Mathers et al. (1977) used a variety of protein supplements and compared nitrogen disappearance from dacron bags to ruminal protein degradation obtained from measurements of nonammonia nitrogen flow to the small intestine. Disappearance values after 4 to 6 hours of incubation gave similar estimates of protein degradation to those obtained measuring nonammonia nitrogen flow. However, dry matter disappearance from the bags at this time ranged from 15 to 80% depending on the supplement.

To obtain accurate estimates of in vivo degradation, both rate of degradation and ruminal retention time of the feed particle must be known. Plotting the log of the proportion of nitrogen surviving in the dacron bag versus time indicates that nitrogen disappears from the bag at two rates, one corresponding to wash out or physical loss from the bag and the other to digestion of a slowly degraded fraction (Mohamed and Smith, 1977). Disappearance of nitrogen during a short incubation period (1 to 2 h) period presumably measures nitrogen solubility while loss at longer incubation times corresponds to microbial degradation of protein. Recently, estimates of protein degradation in the rumen have been calculated using rates of nitrogen disappearance obtained from dacron bags together with rumen retention time (Ørskov and McDonald, 1979). Soybean meal was coated with sodium dichromate to estimate rate of passage of the

supplement. An estimate of 71% rumen degradation was measured in vivo. It was calculated that a ruminal retention time of 12.5 hours when combined with ruminal degradation rate yielded the appropriate value (71%) for degradation of soybean meal.

Factors affecting protein disappearance from dacron bags include pore size, bag size, sample size, and particle size of the tested feedstuff (Mehrez and Ørskov, 1977; Van Hellen and Ellis, 1977; Crawford et al., 1978). Various synthetic fiber materials with pore sizes ranging from 5 microns to 150 microns in diameter have been utilized (Van Hellen and Ellis, 1977; Crawford et al., 1978; Stern et al., 1978; Nocek et al., 1979). Small pore sizes inhibit protozoa from entering the bag while large pore sizes allow finely ground particles to pass out; therefore, a balance must be chosen in selecting a pore size. A large sample size to bag surface ratio limits disappearance of feed material (Uden et al., 1974; Van Hellen and Ellis, 1977; Mehrez and Ørskov, 1977). A critical ratio of sample size to bag size, which when exceeded limits disappearance, varies according to type of feed being fermented.

Protein Degradation Versus Protein Solubility

Determining the degradation of various protein sources in the rumen using microbial markers and re-entrant cannulated animals requires a large input of labor, money, and time. Even in situ studies require access to cannulated animals. Therefore, other laboratory procedures which yield results which are indicative of degradation, have been sought. Protein solubility in mineral solutions has been suggested to be correlated with protein degradation in the rumen (Hendrickx and Martin, 1963; Mertens, 1977; Satter et al., 1977) and has been developed for evaluation of feedstuffs. In Table 2 estimates are provided for ruminal degradation and protein solubility of feeds in various solvents. A

Table 2. ESTIMATES OF DEGRADABILITY AND SOLUBILITY OF PROTEIN IN SELECTED FEEDSTUFFS[a]

Ingredient	Ruminal Degradation %	Solubility %
Concentrates		
Casein	90	93
Cottonseed meal	(60), (80)[b]	7
Soybean meal	39, (55), (60)	13, 6-20, 13-20
Peanut meal	63, (78)	40, 11-37
Sunflower seed meal	(75), 81, 72	30, 24-34, 30-34
Fish meal	10, 29, (31), (60)	11
Barley	(40), (72), (90)	17
Corn grain	(40)	12, 2-11, 15
Corn grain:		
Steam flaked	32, 35	8
Dry rolled	42, 42	12
Acid treated	20	15
High moisture	44	64
Formaldehyde treated		
0	73	16
0.2%	29	4
0.3%	4	3
Forages		
Alfalfa hay	32-46, 58, 59, 60	23, 24-33, 26-28
Red clover		
Immature	66, 73	22-30, 26-28
Wilted	45	
3 wk postwilted	53	
Dry mature	43	
Dried, pelleted	40	
Dried grass	(50)	21-27
Grass silage, unwilted	85	23-61
Corn silage	(40)	26-68

[a]Clark and Crooker (1979).

[b]Values in parentheses are gross estimates.

correlation of approximately 0.45 between degradation and solubility illustrates that these two factors are not highly correlated across feedstuffs. Cottonseed meal and soybean meal are readily degraded but quite insoluble. Although ruminal degradation of casein was approximately equal to its solubility, protein degradation exceeded protein solubility for all other feeds. One exception was ovalbumin which is extremely soluble yet very slowly degraded in the rumen (Mangan, 1972).

For common feedstuffs a correlation coefficient of 0.66 was observed between protein solubility after a 1 h incubation in 10% Burrough's solution and nitrogen disappearance from dacron bags suspended in the rumen for 2 h (Crawford et al., 1978). This is not surprising since protein will be solubilized out of dacron bags. These correlations were improved if the feeds were divided into concentrate, hay and silage groups. Protein solubility also has been correlated to in vitro ammonia release. Hendrickx and Martin (1963) have shown that ammonia release from purified proteins after 6 h was positively correlated ($r = 0.99$) to nitrogen solubility in 10% Burroughs solution. Using various feedstuffs, other workers (Little et al., 1963; Crooker et al., 1978) have not demonstrated as close a relationship of ammonia production at various intervals of incubation with solubility in several different solvents. Differences among feeds in the amount of fermentable carbohydrate may be responsible for the absence of a consistent relationship in such studies.

Pichard and Van Soest (1977) suggested that proteins could be divided into four general categories based on solubility and kinetic studies. These include a soluble protein and NPN fraction, two insoluble fractions, one of which is rapidly degraded and another which is slowly degraded, and an unavailable fraction. It seems reasonable to assume that most soluble protein is degraded. Kinetic studies indicate that most insoluble nitrogen does not escape degradation. The ratio of

the three insoluble fractions, along with their retention times, will influence how closely protein solubility and degradation of a feed are related.

Estimates of rumen degradation of slowly degraded proteins have been reported. These estimates are: meat meal (23-30%), blood meal (18-29%), dehydrated alfalfa (34-63%), corn gluten meal (38-54%) and dried distiller's grains (39-52%) (Waller, 1978; Merchen et al., 1979; Zinn et al., 1981; Loerch, 1982). Rapidly degraded protein sources such as SBM have been reported to be 71-85% degraded in the rumen (Zinn et al., 1981; Loerch, 1982). If degradation is calculated as rate of passage from the rumen divided by rate of passage plus rate of degradation in the rumen (Broderick, 1978), feeding of slowly degraded protein will increase dietary flow to the small intestine compared to feeding SBM. This calculation assumes that rate of degradation within the rumen is not influenced by adaption of microbes to a protein source as suggested by Loerch (1982).

Methods of Ruminal Protein Protection and Their Application

Several physical and chemical treatments have been used to protect proteins and individual amino acids from degradation in the rumen. These methods and their effectiveness have been reviewed by Broderick (1975), Chalupa (1975) and Clark (1975b). The most widely researched method for increasing ruminal protein escape has been heating. Tagari et al. (1962) reported that heating improved the nutritional value of soybean meal for sheep by reducing rumen ammonia levels and urinary N output and increasing N retention.

If ruminal protein protection is to be effective, the protein or amino acid must either be denatured (protein) or chemically bound (amino acid) to a carrier such that rate of degradation in the rumen is reduced. The denaturation or chemical bonding to a carrier must be reversible at the acid conditions of the

abomasum or the material will pass from the animal undigested. Of the methods used to protect proteins, the application of heat is most widely used in feed processing. Research has been reported on the influence of autoclaving and baking various protein sources, primarily soybean meal and cottonseed meal (Danke et al., 1966; Glimp et al., 1967; Hudson et al., 1970; Nishimuta et al., 1972; Sherrod and Tillman, 1962; Thomas et al., 1979b).

Protein sources such as blood meal, corn gluten meal and meat meal as produced commercially are extensively heated to dry the material for storage and handling. Processing of SBM also involves use of heat during the initial flaking of the beans (pre-extraction) and in the final stages for solvent reclamation. Soy flakes after oil extraction contain approximately 35% hexane, 8% water and 1% oil (Mustakas, 1980). Heat is used to remove the hexane, but only low levels of heat are required since hexane has a boiling range of 63-69C (Mustakas, 1980). This heating process is sufficient to inactivate most of the trypsin inhibitor present in raw soybeans (Mustakas, 1980) but it may not be sufficient to protect soy protein from ruminal degradation.

Finley and Friedman (1973) reported that heating increased crosslinking within and between protein molecules. Crosslinking is the result of reactions between the epsilon-amino group of lysine and either carbonyl, carboxyl or amide groups (Bjarnason and Carpenter, 1970; Broderick, 1975; Ferguson, 1975). Broderick (1975) suggested that this chemical modification decreased extent of microbial proteolysis in two ways: (1) the soluble fraction of the protein will be reduced and less protein will be available for microbial hydrolysis and (2) the enzyme reactive sites on the protein will be blocked by chemical rearrangement of the protein. Mahadevan et al. (1980) reported that disulfide crosslinking in proteins was responsible for decreased ruminal degradation, probably due to

blocking of enzyme reactive sites. Whether disulfide crosslinking occurs with thermal denaturation is not known.

Thermal or heat denaturation is reversible if conditions are appropriate and denaturation is not too severe (Lehninger, 1975). Heat denaturation is a problem in processed feeds when proteins are heated in the presence of carbohydrates. This results in the formation of Schiff bases (Broderick, 1975) or the Maillard reaction. The Maillard reaction between a free amino group and the aldehyde group of a reducing sugar has been implicated to be resistant to enzymatic digestion (Pichard and Van Soest, 1977).

The extent of heat damage of protein can be estimated by analyzing feeds for acid pepsin insoluble nitrogen (APIN) or acid detergent insoluble nitrogen (Goering and Van Soest, 1970; Pichard and Van Soest, 1977). The ADIN fraction includes N chemically bound to lignin or cellulose plus N in Maillard products. APIN also has been suggested as a measure of unavailable N (Goering and Van Soest, 1970). This procedure simulates conditions in the abomasum and should provide a more reliable estimate of total N unavailable for digestion since it measures both N resistant to enzymatic degradation plus that bound to the acid detergent fiber (ADF) of feeds.

Chemical treatments which have been employed to reduce ruminal protein degradation include formaldehyde, alcohols and tannins. Formaldehyde treatment has been used most frequently and reacts with free amino groups of lysine and N-terminal amino acid residues forming Schiff's bases and methylene cross links between peptide chains (Fraenkel-Conrat and Olcott, 1948; Broderick, 1975). These bonds reduce solubility of proteins and are stable in the rumen. This binding is reduced under the more acidic environment of the abomasum which allows some of the bound protein to be digested in the small intestine. Some chemical bonds with lysine may be permanent.

Tannins also have been used to render proteins resistant to degradation in the rumen. Hydrolyzable tannins form reversible cross-links with proteins by hydrogen bonding (McLeod, 1974; Ferguson, 1975). Such protein-tannin complexes have reduced susceptibility to deamination by rumen microorganisms but are unstable at gastric pH (Van Buren and Robinson, 1969; Driedger and Hatfield, 1972).

Heat-treatment can also alter degradation of protein in the rumen. Heating hexane-extracted SBM reduces the amount of SBM which is soluble in rumen fluid and most other solvents (Sherrod and Tillman, 1962; Tagari et al., 1962; Little et al., 1963; Glimp et al., 1967; Hudson et al., 1970; Schingoethe and Ahrar, 1979; Thomas et al., 1979a). Protein solubility has been correlated positively with rumen degradation (Crawford et al., 1978; Crooker et al., 1978; Poos-Floyd et al., 1985). These investigators examined several solvents and correlated solubility to in vitro degradation. However, Mahadevan et al. (1980) demonstrated that soluble proteins are degraded at different rates and to different extents by an isolated bacterial protease, so protein solubility alone may be insufficient as a predictor of total ruminal degradation. Mahadevan's work may be questionable since only very low percentage of the test proteins were actually degraded by the isolated protease. Using his value of 6.5 mmoles of amino acids being equal to 1 mg protein, the percentage of serum albumin degraded was only .86% of that incubated with protease isolated from Bacteroides amylophilus. Mangan (1972) demonstrated that ovalbumin, a soluble protein, resists degradation in the rumen, probably because of its cyclic structure which reduces attachment to ruminal microbes which must precede protease attack. Zinn et al. (1981) measured the flow of protein to the small intestine from protein sources with solubilities in a saline solution (10 - 27% soluble N). Ruminal degradation ranged from 30 to 85% and was not correlated with solubility. Thus, solubility of a protein must be used

cautiously in predicting rumen degradation across various sources of protein. But, within a protein type, solubility may predict relative ruminal digestion.

Effect of Altering Protein Solubility on Milk Production by Dairy Cattle

The response of ruminant animals to diets of various protein solubilities and the techniques used to alter solubility have been reviewed previously (Broderick, 1975; Chalupa, 1975; Clark, 1975a; Ferguson, 1975; Huber and King, 1981; Santos et al., 1984). Common methods which have both reduced protein solubility and elicited responses in nitrogen metabolism include formaldehyde treatment (Reis and Tunks, 1969; Peter et al., 1971; Spears et al., 1979; Pankhurst et al., 1980; Crooker et al., 1983; and Brookes, 1984), heat treatment (Sherrod and Tillman, 1962; Tagari et al., 1962; Beever et al., 1976; Ahrar and Schingoethe, 1979; Broderick and Craig, 1980; Mielke and Schingoethe, 1981; Block et al., 1981; Netemeyer et al., 1982; Kung et al., 1983; Vicente et al., 1984; Sahlu et al., 1984; Yang et al., 1984; and Stern et al., 1985) and formulating diets based on components with low solubility (Wohlt et al., 1976; Majdoub et al., 1978; Nocek et al., 1979; Grieve et al., 1980; Cowan et al., 1981; Ørskov et al., 1981; Grummer et al., 1982; Kung and Huber, 1983; Van Dijk et al., 1983; Castle et al., 1983; Erdman and Vandersall, 1983; Ha and Kennelly, 1984; Santos et al., 1984; Higgenbotham et al., 1984; Vandersall and Erdman, 1984). Generally, nitrogen retention has increased when solubility of proteins fed to ruminant animals has been reduced (Sengar and Mudgal, 1983; Kung et al., 1984; and Santos et al., 1984). Often, treatments will increase the quantity of N excreted in feces, but this loss is usually over-compensated by reduced nitrogen excretion by the kidneys (Sengar and Mudgal, 1983).

Most research concerning animal responses to diets varying in protein solubility have used ruminant animals other than lactating dairy cows. With lactating cows, feeding formaldehyde-treated soybean meal (Satter et al., 1970; Clark et al., 1974) or casein (Wilson, 1970; Broderick and Lane, 1978) did not alter milk production or milk composition. In contrast, formaldehyde-treated proteins increased wool growth (Ferguson, 1975) and weight gains in young ruminants (Faichney, 1971; Clark, 1975; Spears et al., 1979; Tamminga, 1979; Thomas et al., 1979b; Spears et al., 1980).

Clark et al. (1974) observed that treating soybean meal with 0.9% wt/wt formaldehyde reduced protein digestibility in the rumen. But, if the level of formaldehyde treatment is excessive, the formaldehyde-protein linkages may not be cleaved under the acidic conditions of the abomasum and protein may not become available for digestion and absorption in the small intestine. Reduced digestibility of casein treated with 0.8% formaldehyde (wt/wt) also may have prevented response in the trial of Broderick and Lane (1978). Ha and Kennelly (1984) concluded from in situ nylon bag studies that formalin-treatment of canola meal reduced both dry matter and nitrogen disappearance. Treatment of soybean meal with formaldehyde in 12% protein diets reduced ruminal NH_3-N concentrations 2 to 3 h post-feeding. Apparent digestibilities of dry matter, organic matter, acid detergent fiber, ether extract and nitrogen-free extract in the total digestive tract were not affected by dietary treatment suggesting that formaldehyde-treated soybean meal was not overprotected and unavailable for animal use.

Formaldehyde treatment of proteins increased milk production in dairy cows (Ferguson, 1975; Mueller et al., 1975; Stobbs et al., 1977; Flores et al., 1979). Brookes (1984) reported that formaldehyde effectively protected casein against rumen degradation for at least 8 hours post feeding. Total nitrogen digestibilities

were similar for the protected and unprotected casein suggesting that the treated casein was digested post-ruminally.

Recent work by Pankhurst et al. (1980), Crooker et al. (1983) and Brookes (1984) suggests that responses to formaldehyde treatment are variable. Pankhurst et al. (1980) in two experiments monitored milk and linoleic acid production by lactating dairy cows fed various forms of formaldehyde protected sunflower seed supplements. No differences in milk production or milk constituents were detected. Soybean meal treated with formaldehyde (0.3% g/100g) inhibited microbial degradation in the rumen of lactating cows but decreased the total tract availability of the soybean meal protein (Crooker et al., 1983). Digestibilities of protein by cows was reduced (62.4 vs. 65.4%) and milk percentage and yield of crude protein were also reduced ($P < .10$) during days 64 to 119 of lactation by formaldehyde treatment of soybean meal. In contrast, Verite and Journet (1977) found that milk, protein, and milk yields were increased by formaldehyde treatment of soybean and rapeseed meals. Sengar and Mudgal (1983) also observed a tendency for milk production to increase with formaldehyde treatment of groundnut cake in lactating Beetal goats.

Wohlt et al. (1976) formulated diets for wethers from common feedstuffs in which either 13 or 35% of the total protein was soluble in a mineral buffer solution. Nitrogen retention and efficiency were increased by reduced protein solubility. Selecting natural feeds of low and high solubility also has been tested with lactating dairy cattle (Braund et al., 1978; Davis, 1978; Majdoub et al., 1978; Grummer and Clark, 1982; Kung and Huber, 1983; Stern et al., 1983; Santos et al., 1984). Majdoub et al. (1978) fed lactating dairy cows two levels of total protein (13 and 15%) with two levels of soluble nitrogen (22 and 42%). Even though diets differed in feed comnposition, they were formulated to contain equal quantities of total digestible nutrients and net energy for lactation. Yields of milk and milk

components were higher ($P < .05$) for the high protein-low solubility diet than for the other three diets during a 9-wk trial. Feeding the low protein-low solubility diet resulted in greater milk yield than feeding the high protein-high solubility diet though the difference was not significant.

Braund et al. (1978) divided 80 cows averaging 31 kg of milk per cow daily into four equal groups to examine the effect of protein solubility on lactational performance. Diets included a negative control (low crude protein 12.6%) and three high protein rations (averaging 18.3% crude protein) varying in protein solubility. The high protein ration containing the highest nitrogen solubility also contained urea. Nutrient composition was identical in all diets except for protein content. Cows fed the high protein ration with the lowest solubility (21.3%) produced more milk than those receiving other three treatments. These researchers recommended that at least 15% but not more than 25% of the total dietary nitrogen should be soluble in the rumen. In fact, they had no treatment with a solubility under 21%.

Davis (1978) conducted a similar trial in which lactating dairy cattle were fed six diets formulated from either natural protein or natural protein plus urea. Protein solubilities ranged from 22.9 to 51.3%. In contrast to trials of Braund et al. (1978), cows fed the diets with the least soluble N produced less milk than cows fed all other diets except for the low protein negative control. Ruminal ammonia levels in the rumen probably were too low for microbial protein synthesis. Lactating cows fed diets containing 30 and 35% soluble nitrogen but no urea produced the most milk. Additional factors such as soluble carbohydrate level, amino acid profile of proteins and nonprotein nitrogen supplies also could influence milk production of lactating cows when ration solubility is varied by selecting specific natural feeds.

Solubility of diet protein has been reduced by exposing soybean meal to moist heat in a cooker-extruder. Ahrar and Shingoethe (1979) fed eighteen cows 9 weeks postpartum soybean meal or soybean meal which had been more extensively heated. In this 16-wk study, no differences in milk production or milk composition were detected, possibly due to the small difference in protein solubility between the two diets (12 vs. 17%) and because the cows were past their peak of lactation.

Grummer and Clark (1982) fed five diets varying in nitrogen solubility to cows in a 20-wk lactation study. Diets ranged from 21.7 to 34.4% soluble nitrogen determined by solubility in .15 M NaCl, .02 M NaOH and 10% Buroughs mineral buffer. Nitrogen solubility of the diets was altered by heat treatment of defatted soybean flakes. The diets provided 85 and 100% of net energy for lactation. Treatments did not significantly increase milk yield or 4% fat corrected milk yield or alter milk composition.

Eighty-four Holstein cows were fed 11.3, 14.5 or 17.5% protein diets with ammoniated versus untreated corn silage and heated or normal soybean meal in a trial by Kung and Huber (1983). Increasing the ration protein percentage increased ($P < .01$) milk yield. Milk production and persistency were lowest for cows fed 11% crude protein corn silage-soybean meal and highest for cows fed 17.5% crude protein ammoniated silage-heated soybean meal diet. Average percent milk fat, milk protein and total solids were not significantly altered by other dietary treatments. Based on these results, it may be concluded that when a diet contains protein with reduced ruminal degradation, more NPN can be used and may be needed to furnish adequate amounts of ammonia in the rumen.

Santos et al. (1984) used four lactating Holstein cows fitted with T-type duodenal, ileal, and rumen cannulae to study the effects of protein source on protein degradation in the rumen and amino acid flow to and absorption from in the small intestine. Ruminal protein degradation was higher for the soybean meal

diet (70%) than for the corn gluten meal (45%), wet brewers grains (52%) and distillers dried solubles (46%) diets. Though the relatively resistant to degradation in the rumen, corn gluten meal and wet brewers grain appeared to be available in the small intestine. Despite the reduced total tract digestibility of the distillers dried grains, the quantities of amino acids disappearing from the small intestine were slightly higher for corn gluten meal, wet brewers grains and distillers dried grains than for soybean meal. It seems unlikely that these three protein sources would result in greater milk yield by lactating cows than soybean meal when fed at normal protein levels.

Past research on circumventing ruminal protein degradation in the rumen for lactating dairy cattle has often increased milk and milk-protein yield in cows fed well-balanced rations. Selecting natural feedstuffs, or heat and chemical treatment to decrease ruminal protein degradation often has improved growth and milk production. Although experimental rations can be formulated to vary only in protein solubility and to contain similar levels of crude fiber, other factors vary. These include levels of soluble carbohydrates, and other nutrients and the amino acid pattern of the diet reaching the small intestine. These would be expected to differ with diet.

To more clearly determine the effect of soybean meal protein heat treatment and solubility on milk production, milk composition and ruminal degradation, the following research trials were conducted. High producing dairy cows were fed diets that differed only in degree of heat treatment of soybean meal. In complementary studies, the extent of ruminal proteolysis, rumen turnover rate and milk production response to heat treatment of soybean meal were examined.

CHAPTER III

IMPACT OF PROCESSING CONDITIONS ON BYPASS POTENTIAL OF N FROM FULL-FAT AND SOLVENT-EXTRACTED SOYBEAN MEAL

Summary

Thirty samples of full-fat and 44% solvent extracted soybean meals (SBM) were extruded under specified manufacturing conditions. These were examined by laboratory and in situ procedures to identify those meals having promise as a ruminal escape protein source for ruminant animals. Extruding conditions for whole soybeans included various combinations of steam turn rate (6, 11, 16), number of holes in an extruder die (336, 588, 840) and feed rate (rpm; 10, 13, 16) and for solvent-extracted meals included steam turn rate (9, 14, 19), feed rate (8, 10, 12) and water percentage (45, 60, 70) with an 840 extruder die. The only production parameter which altered ($P < .01$) N solubility of full-fat SBM was the number of holes in extruder die. Contour plots of response surface indicated that die 840 was most effective. Variations in processing conditions for solvent-extracted SBM resulted in only small numerical differences in nitrogen solubility (5.9 to 10.9%) and pepsin insoluble nitrogen (8.6 to 11.6%). No significant differences in dry matter and nitrogen disappearance were detected ($P > .05$). Several production factors influenced N solubility. These included temperature, water, water x feed, and steam x feed rate. Since the magnitude of change was small with both full-fat soybeans and 44% solvent-extracted SBM, the practical significance of altering processing conditions to increase ruminal escape of these

products appears too small to justify the additional cost.

(Key Words: Soybeans, Processing, N Bypass, Ruminant.)

Introduction

Research efforts in the early sixties demonstrated that under certain conditions animal production can be improved when supply of protein or amino acids to the small intestine is increased (Egan, 1965; Little and Mitchell, 1967; Schelling and Hatfield, 1967). Currently, one of the objectives in protein nutrition of ruminant animals is to increase ruminal escape of high quality feed protein while maximizing bacterial protein supply to the small intestine (Owens, 1978). Manipulating ruminant protein digestion to boost protein escape could permit greater substitution of inexpensive NPN for feed protein. The major animal class which could benefit from increased ruminal protein escape is the high producing dairy cow. Large quantities of amino acids must be absorbed from the small intestine to provide an adequate supply for milk protein synthesis.

Even when the correct feed recommendations are prescribed, high-producing dairy cows may still suffer from a storage of energy and protein since maximum dry matter intake does not occur until after peak milk production. This forces high producing cows to mobilize body stores of fat and protein to meet the deficit. Since body reserves are limited, adding fat and high quality protein that escapes rumen degradation should benefit lactating cows at the peak of lactation and permit diet formulation to lower protein levels for cows at later stages in lactation.

During peak lactation, the quantity of amino acids absorbed from the small intestine may be insufficient to meet the demand for production of milk and thereby limit production. In this instance, providing a protected or slowly

degraded dietary protein to increase the supply of high quality protein to the small intestine should be beneficial. Certain trials have shown that productivity can be increased by heat treating soybean meal used in animal studies (Netemeyer et al., 1982; and Amos, 1980).

The objective of this study was to examine the impact of specific manufacturing processes on the potential ruminal escape of protein from extracted soybean meal and full-fat soybeans. The manufacturing variables tested included die opening, moisture content, steam pressure and feed rate. Meals with the greatest potential for use as a bypass source of high quality protein were identified.

Materials and Methods

Thirty samples of full-fat and solvent extracted soybean meals which were heat treated under controlled manufacturing conditions were examined by laboratory and in situ procedures to identify the potential impact of specific factors on protein and total tract digestibility. Solubility in .15 M NaCl (Waldo and Goering, 1979) was measured and in situ dacron bag procedures were used to evaluate each sample. Decreased solubility and in situ disappearance were used as predictors of potential ruminal protein escape. Pepsin insoluble nitrogen, an index of indigestibility also was measured (A.O.A.C., 1975). Response surface methodology (RSM) was used to determine the optimum combination of manufacturing conditions to minimize each of these variables (SAS, 1979). Conditions tested included three steam turn rates, three different die openings (number of holes in extruder die) and three different feed rates (RPM). Of the 27 potential combinations of factors, fifteen were tested with full-fat soybean meals (Table 1) and fifteen different combinations were tested with solvent extracted meals (Table 2).

For in situ measurement, subsamples weighing approximately 1 g were placed in 8 x 12 cm dacron bags with pore sizes between 50 and 75 microns. Bags were constructed from dacron cloth (100% dacron polyester, R102 Marvelaire White; Erlanger, Blumgart and Co., Inc., 1450 Broadway, New York, New York 10018) due to success in previous studies (Weakley, personal communication). A single piece of dacron cloth measuring approximately 16 x 24 cm was folded in half and double sewn along two of the open edges with polyester thread. The substrate samples were inserted through the top of the bag.

Waterproof glue (Duco cement, DuPont Co., Wilmington, DE 19898) was applied to the sewn area to prevent particulate loss through the needle holes. All cut edges were singed with a hot spatula to prevent fraying in the rumen. Bags had a surface area of approximately 192 cm^2. Prior to filling, bags were machine washed and dried for 24 h at 100C, cooled in a dessicator and weighed. After addition of samples, bags were tied with nylon yarn. Bags were individually tied to the end of a 60 cm yarn cord and grouped by time. Individual bags were labeled with a waterproof marker. Approximately 5 cm from the end of the cords a 30 g steel nut was used to weigh the bags and cord.

Sample bags in duplicate were incubated in the ventral sac of the rumen for 4, 12, and 20 h. Bags were washed under a stream of tap water (approximately 26C) until rinsing water was clear. Washing time averaged approximately 150 sec/bag. Bags were dried in a forced air oven for 48 h at 60C, cooled in a dessicator and weighed.

For in situ incubation, one lactating ruminally cannulated cow approximately 700 kg was fed a diet consisting of 50% concentrate, 30% sorghum silage and 20% alfalfa hay (Table 3). Feed composition was limited to 3% body weight per day in two meals daily (0400 and 1600 hours). To standarize time after feeding effects, bags were introduced together and withdrawn at respective times.

Nitrogen by macro Kjeldahl N analysis, and dry matter determination followed A.O.A.C. (1975) procedures. Disappearance of dry matter (DM) and nitrogen (N) were calculated as follows:

$$\% \text{ DMD} = [\text{initial substrate dry wt} - \text{residual dry wt}] \times 100 / \text{initial substrate dry wt}$$

$$\% \text{ ND} = [\text{initial substrate N content} - \text{residual nitrogen content}] \times 100 / \text{initial substrate N content}$$

Results and Discussion

Manufacturing variables studied with the full-fat soybean experiment included die size, number of turns on the steam valve and feed rate measured in rpm. Nitrogen solubility for the full-fat soybeans ranged from a low of 7.1 for the 840 die size, 16 turns on the steam value and 13 RPM feed rate to a high of 14.4 for the 588-6-10 treatment (Table 4). Only sight differences in dry matter and nitrogen disappearance occurred at any time, though all processed samples were less digested than the control sample after 12 h of digestion. There was a negative correlation between Pepsin Insoluble Nitrogen (PIN) and N solubility (r^2 = - 47; P .01). (Plots vs time and correlations in Appendix A.)

Response surface methodology was used to prepare plots (Figures 1 - 3) to determine the optimum conditions. To minimize nitrogen solubility the optimum combination was the 840 die, 5 to 8 steam value turns with a feed rate of 14 to 16. The only variable contributing significantly to N solubility (Table 5) was the number of holes in the die (P .01). Based on these and other results, the 840 die was used in preparing the meals used in animal feeding studies and in tests with SBM. Additional laboratory analyses of the meals were conducted (Table 6). Values obtained were within expected ranges though extrusion conditions would not be expected to alter nutrient composition. Drops in fiber and ash content and

increases in ether extraction with extrusion were surprising and deserve further attention. These changes indicate that laboratory results vary with feed processing conditions. Whether nutrient availability is similarly altered has not been determined.

Analytical results with extruded 44% protein solvent-extracted soybean are presented in Table 7. The different processing conditions employed resulted in only small changes in the variables measured. Nitrogen solubility ranged from 5.9 to a 10.9 and PIN from 8.6 to 11.6. Stepwise regression revealed that several factors contributed to N solubility. These were: temperature ($P < .01$), water ($P < .01$), water x feed rate ($P < .01$) and steam x feed rate ($P < .01$). The RSM contour plots for the full-fat soybean experiment indicate again that the 840 die was the optimum size. Though statistically significant, numerical differences obtained from these various processing conditions were quite small. A temperature change of 28C changed dry matter and nitrogen disappearance by a mean of only 1 percent point. Though statistically significant ($P < .01$) the practical significance of this change is questionable. On the basis of the laboratory data none of the combination of processing conditions tested would not be expected to increase ruminal escape of SBM. More extreme conditions would be needed. Protein, fiber, calcium, ether extract, and dry matter contents of treated meals are shown in Table 8. Analyses all fall within the respective ranges expected for 44% solvent-extracted SBM. Addition of water, especially with higher steam rates, decreased moisture content of the extruded meal.

In conclusion, neither the full-fat soybean meal nor 44% solvent-extracted meal was sufficiently altered by processing conditions to alter their ruminal escape characteristics. Additional laboratory and pilot-plant scale investigators are needed to determine how more extensive processing would alter the feeding and bypass value of soybean meal. With current manufacturing procedures, it may

not be economically feasible to produce a soybean meal with greater ruminal escape. The addition of new innovative manufacturing procedures, possibly employing added chemicals, might yield a more functional ruminal escape soybean meal which would be more useful than conventionally processed SBM.

Table 1. SAMPLE PREPARATION OF FULL-FAT EXTRUDED SOYBEANS[a]

Die Opening, 1/8" Holes	840	588	336
Steam, Turns	6	11	16
Feed Rate, RPM	10	13	16

Sample No.	Die	Steam	Feed Rate
1	840	16	13
2	840	6	13
3	840	11	10
4	840	11	16
5	588	16	10
6	588	6	10
7	588	16	16
8	588	6	16
9	588	11	13
10	588	11	13
11	336	16	13
12	336	6	13
13	336	11	10
14	336	11	16
15	588	11	13

[a]Test substrates provided by Farmland Industries, Kansas City, Missouri.

Table 2. SAMPLE PREPARATION OF EXTRUDED 44 PERCENT SOYBEAN MEAL[a]

840 Die Opening, 1/8" Holes				
Water, %	45	60	75	
Steam, Turns	9	14	19	Head
Feed Rate, RPM	8	10	12	Temp. °C

Sample No.	Water	Steam	Feed Rate	
1	45	9	10	160
2	75	9	10	160
3	45	19	10	154
4	75	19	10	141
5	45	14	8	138
6	75	14	8	132
7	45	14	12	157
8	75	14	12	160
9	60	9	8	154
10	60	19	8	141
11	60	9	12	163
12	60	19	12	149
13	60	14	10	149
14	60	14	10	154
15	60	14	10	141

[a]Test substrates provided by Farmland Industries, Kansas City, Missouri.

Table 3. INGREDIENT COMPOSITION OF DIET DRY MATTER FOR COW IN IN SITU DIGESTION[a] DACRON BAG EXPERIMENT

Ingredient	IRN	%
Corn, ground	4-21-018	25.0
Soybean meal	5-04-604	11.25
Sorghum, grain	4-04-383	8.75
Molasses, cane	4-04-696	3.75
Dicalcium phosphate	6-01-080	1.0
Salt, trace mineral[c]		.25
Sorghum silage	3-04-468	30.0
Alfalfa hay, chopped	1-00-063	20.0

[a]Contained 14.9% crude protein.

[b]International reference number.

[c]Morton Salt Co., Chicago, IL 60606; contained, as a percentage: NaCl not more than 97%, not less than 92%.

Table 4. DISAPPEARANCE OF DRY MATTER AND NITROGEN (N) FROM DACRON BAGS AND PEPSIN INSOLUBLE N AND N SOLUBILITY OF FULL-FAT EXTRUDED SOYBEANS

Sample No.	Nitrogen[a] Solubility	Dry Matter Disappearance				Nitrogen Disappearance				Pepsin[a] Insoluble Nitrogen
		Exposure Time, Hr.								
		0	4	12	20	0	4	12	20	
	%									
1[a]										
2	7.1	36.6	43.9	58.1	77.2	11.2	17.1	27.2	55.3	9.9
3	8.7	32.0	43.6	57.7	73.2	6.9	18.3	30.3	50.9	10.9
4	7.1	35.4	43.2	56.1	72.3	11.7	17.2	26.7	46.7	9.7
5	11.8	36.3	47.7	60.2	73.3	13.4	22.0	33.6	49.5	9.7
6	14.4	42.3	48.4	64.8	75.9	26.3	18.0	34.7	48.4	8.7
7	11.1	40.3	49.3	60.4	73.1	16.0	20.6	31.2	48.0	9.2
8	8.3	42.9	51.2	63.7	70.6	17.5	24.1	34.7	43.0	9.4
9	8.4	42.7	44.7	56.8	68.7	14.3	17.4	30.1	46.7	10.9
10	10.6	52.4	56.2	59.2	72.7	25.7	33.6	33.5	52.0	10.6
11	12.3	39.9	48.2	59.4	74.7	17.9	24.8	36.2	59.6	10.4
12	12.0	44.6	52.7	64.4	76.1	20.7	24.6	33.9	52.6	9.0
13	11.2	39.9	47.1	58.0	70.1	14.8	22.1	32.6	48.0	10.0
14	13.1	43.9	45.3	57.2	69.7	19.4	22.4	33.1	49.2	12.4
15	9.3	37.1	45.3	58.0	72.4	14.8	18.1	28.6	48.6	7.6

[a]% of total N; sample ground through 2 mm screen.

[b]Sample No. 1 molded prior to analysis.

Table 5. RELATIONSHIP OF PROCESSING CONDITIONS TO NITROGEN SOLUBILITY OF FULL-FAT EXTRUDED SOYBEANS[a]

Parameter	Coefficient	Probability
Die	2.29	.004
Steam	.1125	NS
Rate	- .83	.14
Die^2	- .55	NS
Die Steam*	- .025	NS
$Steam^2$	.845	NS
Die Rate*	.90	NS
Steam Rate*	1.35	NS
$Rate^2$	1.12	NS
R^2 = .88		
CV = 12.99%		

[a]Statistical analysis provided by Farmland Industries, Kansas City, Missouri.

Table 6. LABORATORY ANALYSES OF FULL-FAT EXTRUDED SOYBEANS[a]

Sample No.	Treatment[b]	%						
		Protein	Fiber	Ash	Calcium	Phosphorus	Ether Extract	Dry Matter
1	840/16/13	34.90	5.57	5.54	.48	.50	20.28	87.85
2	840/6/13	37.20	5.40	5.65	.46	.52	21.35	93.15
3	840/11/10	36.20	5.07	8.49	.47	.51	23.36	92.34
4	840/11/16	36.30	9.79	5.45	.49	.51	22.44	92.73
5	588/16/10	38.50	4.87	6.26	.49	.53	19.64	91.9
6	588/6/10	35.80	5.38	6.21	.49	.51	24.80	92.99
7	588/16/16	35.60	5.42	5.79	.43	.54	21.50	93.9
8	588/6/16	38.10	4.66	6.85	.48	.53	23.49	94.21
9	588/11/13	36.20	5.16	5.83	.48	.51	21.43	91.34
10	588/11/13	35.90	6.41	5.78	.49	.51	21.96	91.47
11	336/16/13	37.75	6.03	6.38	.47	.59	22.40	92.74
12	336/6/13	37.85	4.57	6.25	.46	.57	21.06	90.22
13	336/11/10	35.20	5.85	5.71	.52	.54	20.42	93.10
14	336/11/16	36.70	5.29	6.02	.48	.52	21.19	94.26
15	588/11/13	35.30	5.60	6.02	.49	.50	24.75	92.58
16	Untreated[c]	35.30	11.41	7.97	.49	.53	19.27	95.51

[a]Analyses on dry matter basis provided by Farmland Industries, Kansas City, Missouri.

[b]Number of 1/8" holes in die/steam turn, rate/feed rate, rpm.

[c]Composite of above samples prior to treatment.

TABLE 7. DISAPPEARANCE OF DRY MATTER AND NITROGEN (N) FROM DACRON BAGS AND PEPSIN INSOLUBLE N AND N SOLUBILITY OF EXTRUDED 44 PERCENT SOLVENT-EXTRACTED SOYBEAN MEAL

Sample No.	Nitrogen[a] Solubility	Dry Matter Disappearance				Nitrogen Disappearance				Pepsin[a] Insoluble Nitrogen
		Exposure Time, Hr.								
		0	4	12	20	0	4	12	20	
	%									
1	10.6	26.6	35.7	48.0	56.6	6.5	15.0	20.9	27.0	10.7
2	8.3	25.9	34.6	48.9	55.9	6.5	14.1	19.1	33.8	10.6
3	9.4	28.7	38.1	45.0	59.5	10.7	21.3	19.1	39.5	9.5
4	8.4	28.5	33.2	45.9	59.6	8.2	9.0	19.7	34.8	9.8
5	8.2	34.2	35.4	42.7	59.8	9.6	11.8	25.0	27.5	11.6
6	7.0	25.1	33.6	46.1	60.7	4.8	13.6	21.2	36.1	12.3
7	10.0	28.5	34.9	56.1	55.5	7.8	13.9	30.1	25.9	10.8
8	10.1	21.7	35.8	49.7	56.9	5.0	15.0	20.4	30.2	10.9
9	8.7	27.5	36.1	44.9	58.1	7.7	15.5	17.5	31.8	9.7
10	7.5	25.6	29.3	37.9	57.1	3.7	6.8	17.4	34.6	10.4
11	10.8	33.3	33.6	44.0	52.6	7.3	11.3	22.3	27.4	9.7
12	10.9	28.7	35.4	46.3	57.4	14.1	18.2	20.7	31.7	8.6
13	7.6	32.0	33.2	45.6	52.8	7.6	9.7	24.4	27.5	9.3
14	10.1	32.0	34.6	42.7	53.8	7.8	13.6	20.0	30.3	10.0
15	5.9	30.8	36.3	44.6	57.4	5.4	13.0	14.3	28.8	11.3

[a]% of total N; sample ground through 2 mm screen.

Table 8. LABORATORY ANALYSES OF EXTRUDED 44 PERCENT SOLVENT-EXTRACTED SOYBEAN MEAL[a]

Sample No.	Treatment[b]	%					
		Protein	Fiber	Calcium	Phosphorus	Ether Extract	Dry Matter
1	45/9/10	53.68	2.29	.75	.88	1.87	98.21
2	75/9/10	51.72	3.28	.62	.84	1.96	94.79
3	45/19/10	53.51	2.38	.59	.84	1.31	97.35
4	75/19/10	51.21	2.99	.61	.81	1.39	93.29
5	45/14/8	54.13	2.67	.52	.79	2.62	97.68
6	75/14/18	48.83	3.69	.52	.72	1.81	98.88
7	45/14/12	54.48	1.72	.61	.86	1.66	98.96
8	75/14/12	52.56	3.13	.60	.83	1.52	95.36
9	60/9/8	53.30	2.65	.50	.79	1.56	98.53
10	60/19/8	47.76	2.73	.60	.77	1.59	92.85
11	60/9/12	53.07	2.14	.53	.79	1.54	97.14
12	60/19/12	51.58	3.66	.59	.80	1.27	93.54
13	60/14/10	53.28	3.39	.55	.80	2.00	97.15
14	60/14/10	53.52	2.68	.56	.82	1.45	96.02
15	60/14/10	53.00	3.37	.61	.84	3.37	97.16
16	Untreated[c]	49.06	3.68	.30	.67	1.70	90.07

[a]Analyses on dry matter bases provided by Farmland Industries, Kansas City, Missouri.

[b]Water percentage/steam turn, rate/feed rate, rpm with die size 840.

[c]Composite of above samples prior to treatment.

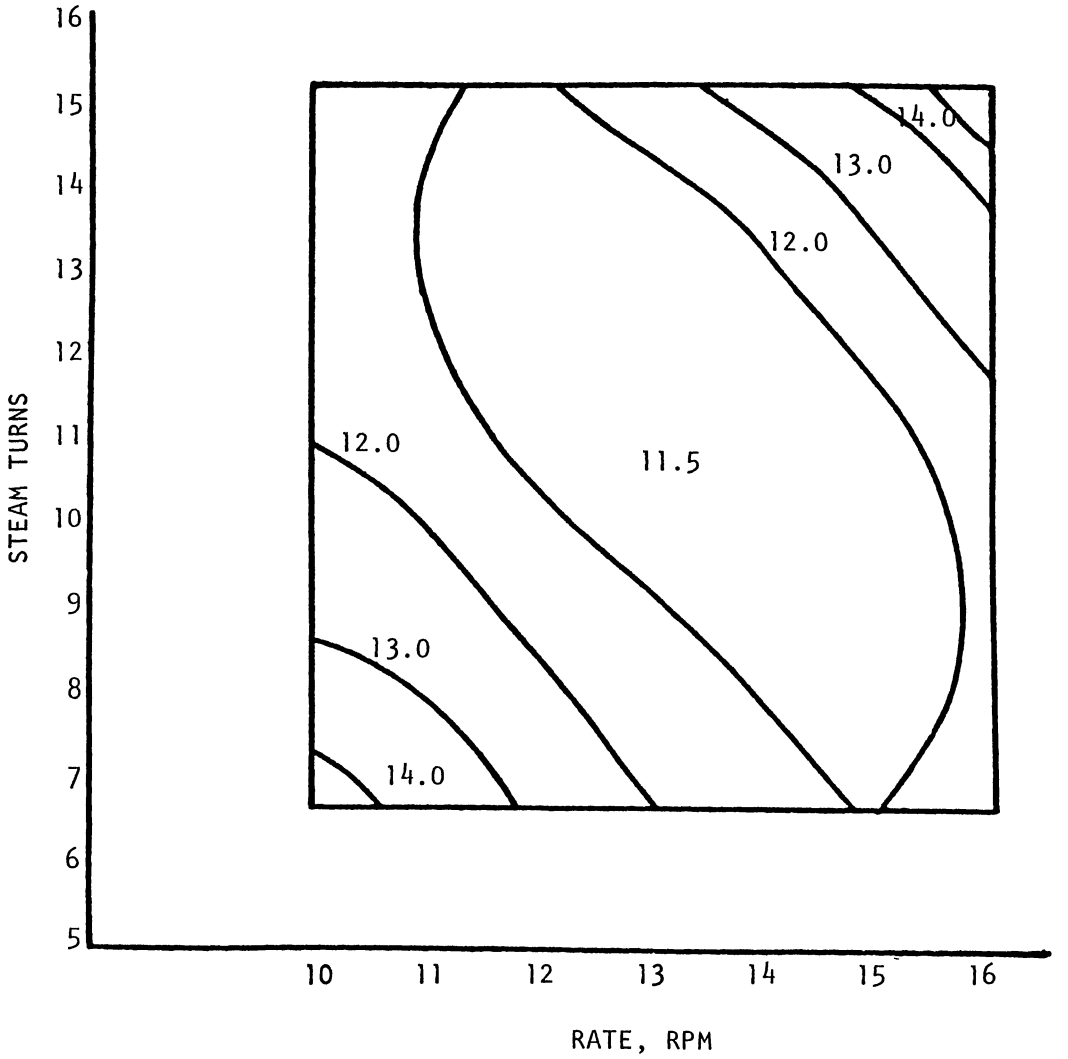

Figure 1. The Influence of Extruding Full-Fat Soybeans on Percent Estimated Nitrogen Solubility
Die = 336 (Contour Plots Provided by Farmland Industries, Kansas City, MO)

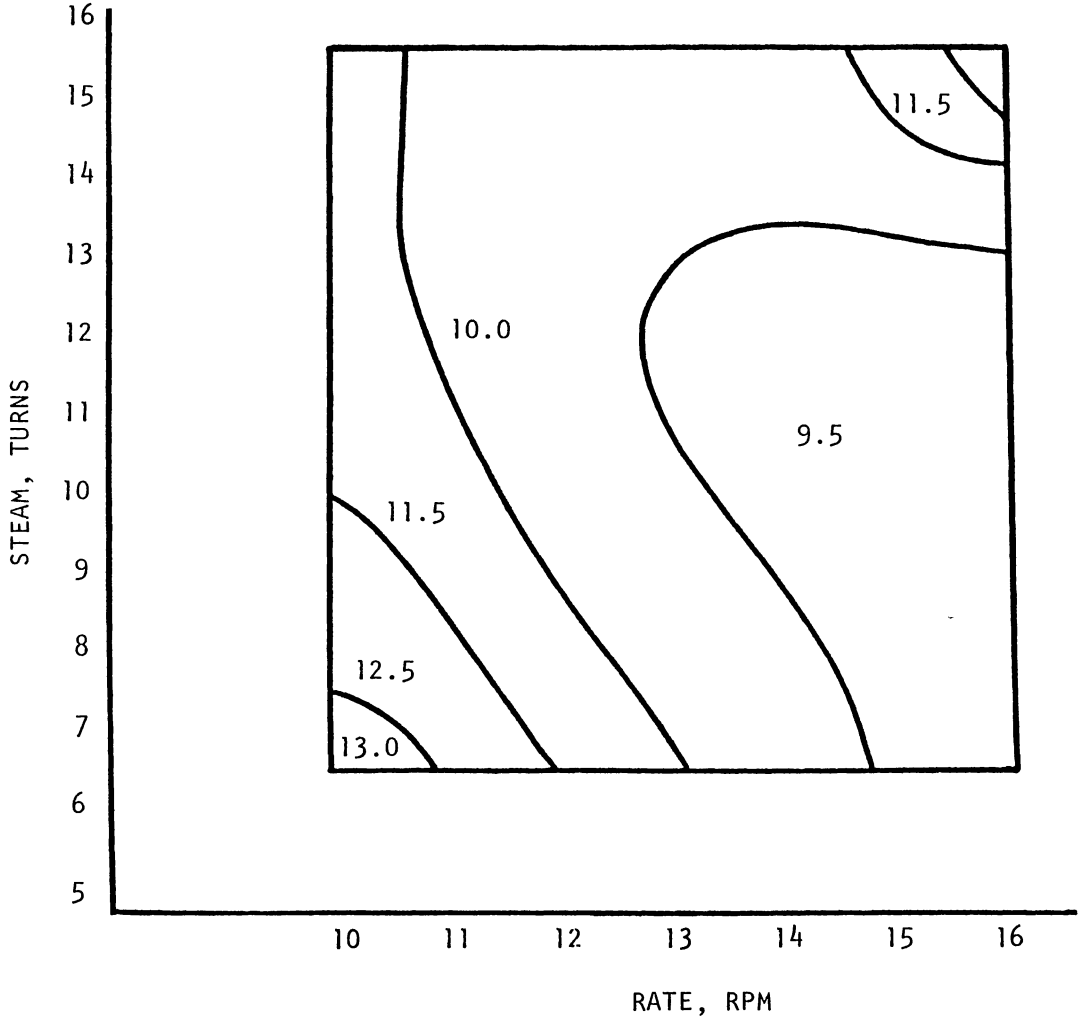

Figure 2. The Influence of Extruding Full-Fat Soybeans on Percent Estimated Nitrogen Solubility Die = 588 (Contour Plots Provided by Farmland Industries, Kansas City, MO)

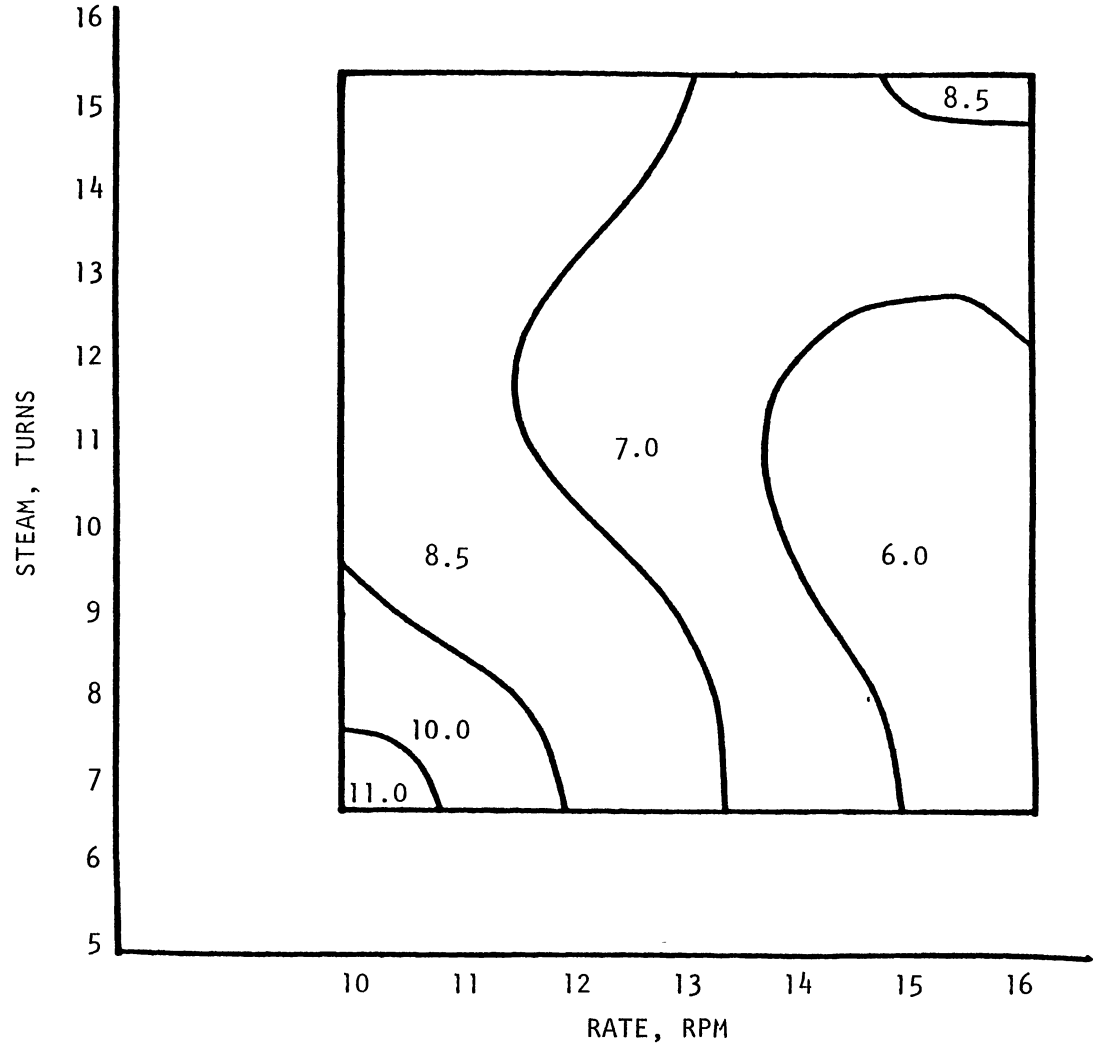

Figure 3. The Influence of Extruding Full-Fat Soybeans on Percent Estimated Nitrogen Solubility Die = 840 (Contour Plots Provided by Farmland Industries, Kansas City, MO)

CHAPTER IV

FEEDING VALUE OF HEAT-TREATED SOYBEAN MEAL FOR LACTATING DAIRY COWS IN A SWITCHBACK EXPERIMENT

Summary

Twenty-four lactating dairy cows (8 to 9 wks postpartum) were utilized in a switchback feeding trial to evaluate the application of heat-treatment of soybean meal (SBM) on animal performance. Feed grade soybean meal with a protein dispersion index of 40 (PDI-40) and SBM which had been more extensively heated (PDI-10) were compared. Diets contained either feed grade SBM, heat-treated SBM (HSBM) or HSBM with 0.3% urea added. SBM protein accounted for approximately 41% and 45% of total protein intake for cows fed the feed grade SBM and HSBM, respectively. Protein intake averaged 99, 98 and 114% of NRC (1978) feeding standards during feeding periods for SBM, HSBM and HSBM plus urea. Dry matter intake and milk yield and composition were similar for all treatments though cows receiving the HSBM tended to gain body weight. Production efficiency (milk/feed intake) was similar for the three treatments (1.38, 1.37 and 1.38). Rumen ammonia levels were elevated in the HSBM plus urea group (11.8 mg/dl versus 8.8 mg/dl and 8.9 mg/dl for SBM and HSBM cows). Heat treatment of soybean meal in this experiment did not improve its feeding value for lactating cows. Failure of heat treatment to affect ruminal ammonia values or on in situ digestion rate infers that the degree of heat-treatment used in this study did not reduce ruminal degradation of SBM.

(Key Words: Soybean meal, Processing, Protein, Ruminants.)

Introduction

High quality feed proteins can be used more efficiently for milk production when a large portion of the protein is protected from ruminal degradation yet is digested in the small intestine. When proteins or amino acids have been infused abomasally, milk production often has been increased (Griel, 1968; Bishop, 1971; Schwab et al., 1976; Clark, 1975). Production also has been increased by formaldehyde treatment (Reid et al., 1971; Mueller et al., 1975; Verite et al., 1977), and by diets formulated to contain less of the total protein as soluble protein (Braund, 1978; Majdoub et al., 1978; Mielke and Schingoethe, 1979; Block et al., 1980; Netemeyer et al., 1982). Protein degradation in the rumen is partially related to solubility (Glimp et al., 1967; Little et al., 1963; Poos-Floyd et al., 1984). Consequently, reducing the solubility of proteins may improve the quantity of protein available to the small intestine for digestion and absorption and could potentially increase milk yield.

Increased ruminal escape of protein is most desirable when protein demand is highest as is the case for young growing ruminants and for high producing dairy cows early in lactation as reviewed by Broster (1972), Clark and Davis (1980) and Huber and Kung (1981). Heat treatment has been reported to reduce protein solubility and microbial degradation of SBM (Netemeyer et al., 1982) and may shift the site of digestion. Unless increased production is obtained from heat treatment of the protein, such alteration is useless. When ruminal protein escape is increased, ruminal bacteria may be potentially starved for ammonia. The addition of urea to the diet could meet this need.

The objective of this study was to measure milk production response to heat treatment of SBM with or without addition of urea.

Materials and Methods

Heat-treated soybean meal (SBM) and conventionally processed SBM were supplied by a commercial manufacturer[1] to compare their feeding value for lactating dairy cows. Both meals were processed by conventional procedures through to the oil extraction step. Following extraction, the solvent was removed by a flash desolventizing method involving 3 to 10 seconds of exposure to superheated (176C) hexane vapor. Meals were then subjected to temperatures ranging from 79 to 115C and up to 704 g/cm^2 of steam. SBM retention time in the cooker unit was approximately 30 minutes. Meals were further characterized by measuring the Protein Dispersibility Index (PDI, Paulsen; 1968). PDI is a measurement of the degree of heat treatment as described by A.O.C.S. (1969). The greater the heat treatment, the lower the PDI. Control SBM had a PDI of 40 and heat-treated SBM had a PDI of 10. Soybean meals were further characterized by the in situ dacron bag procedure and evaluated using lactating dairy cows in a feeding trial.

In Situ Experiment

An in situ dacron bag procedure was employed to compare nitrogen and dry matter disappearance of soybean meals designated PDI-10 or PDI-40. Substrate samples weighing approximately 7 grams were placed in 17 by 9 cm bags with pore size between 50 and 75 microns. Bags were made from dacron cloth (100% dacron Polyester, R102 Marvelaire White; Erlanger, Blumgart and Co., Inc., 1450 Broadway, New York, New York 10018) based on success in previous studies (Weakley, personal communication). Bags were constructed from a single piece of

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[1]Farmland Industries, St. Louis, MO.

material measuring approximately 17 x 18 cm that was folded in half and double sewn along two of the open edges with polyester thread. The top was left open for sample insertion into the bag.

Water-proof glue (Duco cement, DuPont Co., Wilmington, DE 19898) was applied to the stitched area to prevent loss of small particles through the needle holes. All cut edges were singed with a hot knife to prevent fraying in the rumen. The constructed bags had a surface area of approximately 306 cm^2. Prior to filling, bags were machine washed and dried for 24 hrs at 100C, cooled in a dessicator and weighed. After addition of samples, bags were tied with nylon yarn. Bags were individually tied to the end of a 60 cm yarn line and grouped by time. Individual bags were labeled with a waterproof marker. Approximately 5 cm from the end of the line, a 30 g steel nut was tied to weigh the bags down.

Three substrates were investigated. These included two soybean meals received from the commercial manufacturer plus a feed mill source of soybean meal (FSBM) obtained from the University feed mill. The two meals supplied by the manufacturer were identified by manufacturing treatment, i.e., CSBM (conventionally processed SBM) and HSBM (heat treated SBM).

Sample bags in duplicate were incubated in the ventral sac of the rumen for 4, 12 and 20 h. Bags were introduced at the same time and retrieved 4, 12 and 20 h later. Bags were washed under a stream of tap water (approximately 26C) until the rinsing water was clear. Washing time averaged approximately 150 sec/bag. Bags were dried in a forced air oven for 48 h at 60C, cooled in a dessicator and weighed.

For incubation of samples in bags, one lactating ruminally cannulated cow weighing approximately 700 kg was fed a diet consisting of 60% concentrate, 28% sorghum silage and 12% prairie hay (Table 1). Feed was available ad libitum (3%

DM body wt). Fresh feed was fed three times each day at 0300, 1100 and 1900 hours to stabilize ruminal conditions.

Nitrogen, by macro Kjeldahl N analysis and dry matter determination followed A.O.A.C. (1975) procedures. Disappearance of dry matter (DM) and nitrogen (N) were calculated as follows:

$$\% \text{ DMD} = [\text{initial substrate dry wt} - \text{residual dry wt}] \times 100 / \text{initial substrate dry wt}$$

$$\% \text{ ND} = [\text{initial substrate N content} - \text{residual nitrogen content} \times 100 / \text{initial substrate N content}]$$

Lactation Experiment

The two soybean meals were included in the concentrate portion of the complete diets for lactating cows (Table 2). The concentrate portion of the diet was mixed fresh each week of the study. Diets contained either feed grade SBM (PDI-40), heat treated SBM (PDI-10) or heat-treated SBM with .3% added urea. Diets were formulated to provide 1.7 Mcal net energy for lactation (NE_l) per kg of dry matter. Diet dry matter consisted of 60% grain mix, 28% sorghum silage and 12% sudangrass hay.

Prior to initiating the study, twenty-four cows (20 Holsteins, 4 Aryshires) were challenge fed a 60:40 concentrate to forage ration for a period of 2 to 4-wk and cows were started on test diets from 8 to 9-wk postpartum. At the end of the adjustment period cows were randomly assigned to treatments employing a Lucas switchback design (Lucas, 1956) with 4-wk periods. To minimize carryover effects, the data collected during the first week of each period were not included in calculations.

Feed provided was limited based on size of cow, age and production during the preliminary period. Feed allowance was decreased by 5% at the end of each period based on the anticipated decrease in milk production over time, though diets were formulated to provide 85% of the protein specified by the National Research Council (NRC, 1978). Calculating back from production, protein intake averaged 92, 98, and 110% of listed requirements during the first, second and third experimental periods. Decreasing the feed allowance by only 5% at the start of the second and third 4-wk periods was not sufficient to maintain protein intake at the desired level since milk production persistency and yield was less than expected. Over the entire experiment, protein intake averaged 99, 98 and 104% of NRC requirements for the regular soybean meal, heat-treated SBM and heat-treated plus urea diets, respectively.

Cows were fed equal amounts twice daily of the allotted concentrate and sorghum silage portions of the total ration. Once daily, cows were individually fed their allottment of sudangrass hay. Cows were weighed on three consecutive days at the end of the preliminary period and at the end of each experimental period. Each day that feed was refused, 10% of the weighback was collected and refrigerated. At the end of each week, weighbacks were composited and sampled. Composite feed and ort samples were dried to a constant weight at 40C, ground in a Wiley mill (2mm screen) and sampled for dry matter and total protein determinations (A.O.A.C., 1975).

Samples of the concentrate mixtures, sorghum silage and sudangrass hay also were collected each week and analyzed for dry matter and total protein. Milk yields were recorded twice daily and samples were collected from four consecutive milkings each week, composited by volume and analyzed for milk fat and total protein. A Mark II Milk-O-Tester (A/S N. Foss Electric, Denmark) was

used to measure milk fat. Milk protein percentages were determined during the fourth week of each period by the Kjeldahl method (A.O.A.C., 1975).

Ruminal fluid samples were collected during the last three days of each period by stomach tube at 2 h after feeding. Samples were strained through four layers of cheesecloth, acidified by addition of 1 ml 20% H_2SO_4 per 50 ml strained fluid, and analyzed for ammonia-N content (Chaney and Marback, 1962).

Results and Discussion

In Situ Experiment

The extent of heat-treatment and manufacturing conditions presumably affected the outcome of this experiment. The solubility of nitrogen in .15 M NaCl was 7.6% of total nitrogen in the more extensively heat-treated soybean meal compared with 13.9% for the regular soybean meal. These differences are much smaller than a previous trial (Netemeyer et al., 1982) in which nitrogen (N) solubility was reduced from 24.6% of total N to 8.1% by heat treatment. *In situ* disappearance of dry matter (DM) and nitrogen (N) from CSBM and extra heat SBM (HSBM) were not significantly altered ($P > .05$) by heat treatment (Table 3), although DM and N disappearance rates tended to be higher for HSBM than FSBM or CSBM. Hence, the heat treatment might not be expected to reduce ruminal degradation of protein. Such variations in soybean meal solubility due to manufacturing and heat treatment conditions must be controlled to provide a consistent animal response. PDI alone appears inadequate as a measure of *in situ* disappearance rate.

Lactation Experiment

Dry matter intakes (Table 4) by cows were not different ($P > .05$) for soybean meal treatment groups. Soybean meal protein provided 41% of the total protein

intake of cows fed the untreated soybean meal and 45% of the total protein intake of cows fed HSBM. Soybean meal protein in the HSBM plus-urea ration accounted for 36% of the total crude protein. In all groups, soybean meal protein was considered to be at a high enough percentage of the total protein to influence production though protein intakes were 92, 98 and 110% of that listed by the NRC (1978) as being required for the levels of production obtained. Urea was added to the heat-treated SBM to provide additional available N in case heat treatment reduced ruminal ammonia availability which could potentially starve ruminal microbes.

Milk yield and composition were not affected by alteration of dietary protein (Table 5). This result contrasts with previous findings in which milk yield of cows fed more extensively heated soybean meal was higher than that of cows fed regular soybean meal (Netemeyer et al., 1982). Efficiency of production milk/kg DM consumed in their trial was significantly improved ($P > .05$) in the lower protein group receiving 94% of NRC (1978) requirements for protein. In this trial, dietary crude protein intake for the heat-treated soybean meal group averaged 98% of NRC requirement.

Ahrar and Schingoethe (1979) reported that heat-treatment of SBM increased milk production slightly during the first 8-wk of lactation when protein intake was limited but not in the latter part of lactation when protein intake exceeded requirements. Hence, stage of lactation may alter response. In a switchback experiment by Mielke and Schingoethe (1981) milk yield of cows past peak production also was not altered by heat treatment of SBM.

One can examine these results by period, as well. During the first period of this trial, when protein intake averaged only 92% of requirements, milk yields, adjusted by covariance analysis for differences in pre-trial yields, were 26.7, 27.8 and 26.2 kg/day for the regular soybean meal, heat treated soybean meal and heat

treated soybean meal plus urea groups, respectively. Though differences were not significantly different, treatment trends support the concept that heat treatment was more effective early in lactation when protein requirements would be higher.

Differences in body weight change between SBM treatment groups were not statistically different ($P > .10$). During early lactation, lactating cows mobilize protein reserves to meet the protein demands for milk production. Repletion of these reserves should be more rapid if protein quality or quantity is increased. In 1985 Ørskov observed that in experiments conducted with cows maintained by intra-gastric infusion early in lactation, an increase in the supply of protein to the intestines caused no response in blood insulin. Later in lactation, insulin responses were apparent. Increased insulin would be expected to increase weight gain. Ruminal ammonia concentrations (Table 4) were not altered by heat treatment of SBM but were increased by addition of urea to the diet. With all diets, ruminal ammonia concentrations at the times sampled exceeded those generally considered necessary to maximize microbial protein synthesis (Satter and Roffler, 1975). Ruminal ammonia concentrations for the PDI-10 and PDI-40 diets were similar. If degradation of dietary protein in the rumen was reduced by heat treatment, ruminal ammonia level should be reduced. Since it was not reduced, one might suspect that heat treatment did not greatly alter ruminal protein degradation of SBM.

In summary, milk yield by cows in this trial was not increased by feeding a more extensively heated SBM. Ruminal escape was not increased by heat treatment since ruminal ammonia concentrations were not altered. The control soybean meal in this study had a relatively low solubility in saline solution. Though solubility was slightly reduced by heat treatment, extent of ruminal proteolysis as measured *in situ* was decreased. Complete *in vitro* and *in situ* appraisal of responses in protein supplements to heat-treatment and

manufacturing conditions should precede extensive and expensive lactation trials with treated materials.

Table 1. INGREDIENT COMPOSITION AND PROTEIN CONTENT OF DIET USED FOR COWS IN IN SITU DACRON BAG EXPERIMENT[a]

Ingredient	IRN[b]	%
Corn, ground	4-21-018	30.0
Soybean meal	5-04-604	13.5
Sorghum, grain	4-04-383	10.5
Molasses, cane	4-04-696	4.5
Dicalcium phosphate	6-01-080	1.2
Salt, trace mineral[c]		.3
Sorghum silage	3-04-468	28.0
Prairie hay	1-03-191	12.0
Protein, % of DM		13.6

[a]Dry matter basis

[b]International reference number

[c]Morton Salt Co., Chicago, IL 60606; contained as a percentage: NaCl not more than 97%, not less than 92%.

Table 2. INGREDIENT COMPOSITION AND CHARACTERISTICS OF DIETS[a]

Item	IRN[b]	Treatment		
		SMB	HSBM	HSBM+Urea
Ingredients, %				
Corn, ground	4-21-018	47.4	47.4	47.1
Soybean meal (PDI-40)	5-04-604	11.1	--	--
Soybean meal (PDI-10)	5-04-604	--	11.1	11.1
Urea		0	0	.3
Dicalcium phosphate	6-01-080	.6	.6	.6
Limestone	6-02-632	.6	.6	.6
Salt, trace mineral[c]		.3	.3	.3
Sorghum silage	3-04-468	28.0	28.0	28.0
Sorghum sundangrass	3-04-499	12.0	12.0	12.0
Protein, % of DM		14.4	14.2	15.1
SBM-N solubility in .5M NaCl		13.9	7.6	--
NE_{ℓ} (MCal/kg of DM)		1.70	1.70	1.70

[a]Dry Matter Basis.

[b]International reference number.

[c]Morton Salt Co., Chicago, IL 60606; contained as percentage: NaCl not more than 97%, not less than 92%.

Table 3. DISAPPEARANCE OF DRY MATTER (DM) AND NITROGEN (N) FROM SOYBEAN MEAL TYPES (SBM) PLACED IN DACRON BAGS AND SUSPENDED IN THE RUMEN

	Exposure Time, h	SBM Type		
		FSBM	HSBM	CSBM
Dry matter	0	28.4	26.6	23.9
Disappearance, %[a]	4	35.3	35.1	34.9
	12	45.6	48.6	48.3
	20	69.0	69.6	69.6
Residue slopes,				
%/h[b]		- 2.1	- 2.2	- 2.2
%/h[c]		- 4.6	- 4.7	- 4.8
Nitrogen	0	6.2	12.6	8.5
Disappearance, %[a]	4	17.4	18.8	11.0
	12	29.5	32.4	24.1
	20	49.8	64.5	48.6
Residue slopes,				
%/h[b]		- 2.0	- 2.9	- 2.4
%/h[c]		- 3.1	- 4.3	- 3.4

[a]Each value is the mean of two observations.

[b]Linear

[c]Logarithmic

Table 4. TOTAL FEED AND TOTAL PROTEIN INTAKE PERCENT CONCENTRATE AND SOYBEAN PROTEIN OF TOTAL DIET, AND RUMEN AMMONIA AND WEIGHT CHANGE, OF COWS FED HEAT TREATED SOYBEAN MEAL

Item	Treatment			
	SBM	HSBM	HSBM+UREA	SE
Total dry matter intake, kg/day	17.05	17.15	17.01	.26
Total protein intake, kg/day	2.46	2.43	2.57	.08
Rumen ammonia, mg/dℓ	8.8	8.9	11.8	.04
Soybean protein/total protein intake, %	41.0	45.0	36.3	--

Table 5. MILK YIELD AND MILK COMPOSITION OF COWS FED SOYBEAN MEALS CLASSIFIED ACCORDING TO THE PROTEIN DISPERSION INDEX

Item	Treatment			
	SBM	HSBM	HSBM+UREA	SE
Milk, Kg/Day	23.6	23.5	23.4	.29
Fat, %	3.84	3.80	3.85	.05
Protein, %	3.12	3.11	3.09	.06
FCM	24.8	22.8	22.9	.02
Milk/Feed DM	1.38	1.37	1.38	.01
Body Weight Change, kg/wk	- .07	+ 2.03	- .03	.43

CHAPTER V

INFLUENCE OF HEAT TREATMENT OF SOYBEAN MEAL FOR LACTATING DAIRY COWS IN A CONTINUOUS FEEDING TRIAL

Summary

Forty lactating dairy cows (4 weeks postpartum) were used in a continuous feeding trial to study soybean meal subjected to extensive heating during processing (HSBM) versus regularly processed soybean meal. Nitrogen solubilities of the meals were 11.2 and 19.3% of total nitrogen and rates of in situ N disappearance were 2.8 and 2.3% h, respectively. Cows were paired within two production groups (31 to 38 kg/day and 24 to 30 kg/day) based on milk yield during the preliminary period and were fed a 60:40 concentrate to forage ration composed of corn grain, sorghum silage and prairie grass hay. Soybean meal protein provided approximately 53% of the total protein consumed by the high production group and 47% for the lower production group. Dry matter intake, milk yield and composition were similar within production groups, but there was a trend toward higher milk yield by high producing cows fed HSBM (33.5 versus 31.0 kg/day). No production response was observed with the lower production cows (25.1 versus 25.9 kg/day). Weight change was positive and similar for cows fed both meal types.

(Key Words: Soybean meal, Processing, Protein, Ruminants.)

Introduction

Demands for nutrients for milk synthesis are especially high for dairy cows during the first 8 to 10 weeks of lactation. During this time, most cows are not able to consume enough dry matter to support maximum milk production (Bines, 1976; Clark and Davis, 1980; Huber and Kung, 1981) which results in mobilization of energy and protein reserves from the body (Botts et al., 1979). Cows should be most responsive during this period to an improved protein status. Alternative sources of protein have elicited improvements during this period (Grummer and Clark, 1982; Santos et al., 1984). One avenue to improve protein status is to treat soybean meal to increase the fraction of protein which escapes ruminal destruction without reducing protein digestion in the small intestine (Crooker et al., 1983; Brookes, 1984; Vicente, 1984; Sahlu, 1984).

Heat treatment of high quality proteins can reduce protein solubility and increase performance of growing and lactating ruminants (Glimp et al., 1967; Nishimuta, et al., 1974; Schingoethe and Ahrar, 1979) but responses have not been observed consistently (Chapter IV). The objective of this study was to compare production of lactating cows fed regular processed soybean meal with production of cows fed more extensively heat-treated soybean meal. Experimental designs may alter sensitivity of this comparison since protein requirements of lactating cows decrease rapidly as milk production declines. Hence, a continuous feeding study was used in this study.

Materials and Methods

Soybean meals subjected to conventional processing and extra heat treatment were supplied by a commercial processor.[1] The two soybean meals

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[1]Farmland Industries, St. Louis, MO.

were manufactured by commercial methods through the oil extraction step. Following extraction, the solvent was removed by flash desolventizing involving 3 to 10 seconds of exposure to superheated (177C) hexane vapor. Cooker temperature ranged from 79 to 116C with a maximum steam pressure of 704 g/cm^2. Meals were subjected to approximately 30 minutes of processing in the cooker unit. Meals were classified by the processor as to the Protein Dispersibility Index (PDI, Paulsen, 1968). Conventionally processed SBM (CSBM) had a PDI index of 40 whereas the more extensively heated SBM (HSBM) had a PDI value of 10. The value of extra heat treatment was determined by the in situ dacron bag procedure and further examined in a continuous feeding trial with lactating dairy cows.

In Situ Experiment

A dacron bag procedure was used to compare dry matter (DM) and nitrogen disappearance of the feed grade (FSBM), CSBM and HSBM. Feed grade SBM was obtained from the University feed mill for comparison with the two meals received from the commercial manufacturer. Approximately 7 g of SBM were placed in each 17 by 9 cm bag with pore sizes ranging from 50 to 75 microns. Bags were constructed from dacron cloth (100% dacron polyester, R102 Marvelaire White; Erlanger, Blumgart and Co., Inc., 1450 Broadway, New York, New York 10018). A single piece of dacron cloth measuring approximately 17 x 18 cm was folded in half and double sewn along two of the open edges with polyester thread. The substrate samples were inserted through the top of the bag.

Waterproof glue (Duco cement, DuPont Co., Wilmington, DE 19898) was applied to the sewn area to prevent particulate loss through the needle holes. All cut edges were singed with a hot spatula to prevent fraying in the rumen. Bags had a surface area of approximately 306 cm^2. Prior to filling, bags were machine

washed and dried for 24 hrs at 100C, cooled in a dessicator and weighed. After addition of samples, bags were tied with nylon yarn. Bags were individually tied to the end of a 60 cm yarn cord and grouped by time. Individual bags were labeled with a waterproof marker. Approximately 5 cm from the end of the cords a 30 g steel nut was used to weight the bags and cord.

Sample bags in duplicate were incubated in the ventral sac of the rumen for 4, 12, and 20 h. Bags were washed under a stream of tap water (approximately 26C) until rinsing water was clear. Washing time averaged approximately 150 sec/bag. Bags were dried in a forced air oven for 48 hours at 60C, cooled in a dessicator and weighed.

For in situ incubation, one lactating ruminally cannulated cow approximately 700 kg was fed a diet consisting of 60% concentrate, 28% sorghum silage and 12% prairie hay (Table 1). Feed intake was high (3% of body weight daily) and the cow was fed three times daily at 0300, 1100 and 1900 hours to stabilize ruminal conditions.

Nitrogen by macro Kjeldahl N analysis, and dry matter determination followed A.O.A.C. (1975) procedures. Disappearance of dry matter (DM) and nitrogen (N) were calculated as follows:

$$\% \text{ DMD} = [\text{initial substrate dry wt} - \text{residual dry wt}] \times 100 / \text{initial substrate dry wt}$$

$$\% \text{ ND} = [\text{initial substrate N content} - \text{residual nitrogen content} \times 100 / \text{initial substrate N content}]$$

Lactation Trial

Forty lactating dairy cows were used in the continuous feeding trial to compare (1) solvent extracted soybean meal (CSBM) with (2) soybean meal subjected to more extensive heating during processing (HSBM). These two

products were mixed into the concentrate portion of diet prior to combining the complete diet ingredients (Table 2). Cows were divided by production level into two groups and fed two levels of protein (Table 3) using each of the two protein sources. Ration dry matter consisted of 60% concentrate mix, 28% sorghum silage and 12% prairie hay. Test proteins comprised approximately 53% of the total dietary protein for the high production group and 47% of the total dietary protein for the lower production group of cows in this study but calculated energy content (NE_l) was constant. The concentrate to forage ratio was adjusted to minimize overfeeding of protein as milk production declined over the lactation period.

Rations were formulated so that protein intake would be sufficient to meet 90% of the crude protein specified by the 1978 National Research Council (NRC) feeding standard (Table 4) for all cows. The higher protein diets were fed to cows initially producing 31 kg or more of milk daily, whereas the lower protein diets were fed to cows with lower production. For the higher producing cows, crude protein intakes exceeded NRC (1978) recommendations, especially during the second 4-wk period of the trial (Table 4). This was because milk yield declined faster than anticipated for cows fed the higher protein ration.

Approximately 4-wk postpartum, cows were adjusted to a typical dairy ration of concentrates, sorghum silage and prairie hay with a 60:40 concentrate to forage ratio and paired within two production groups. The higher production group consisted of cows producing over 31 kg milk/day during the 5 days prior to initiating the study whereas cows in the lower group produced 24 to 30 kg milk/day during this period. For treatment comparisons, production was measured weekly during the 16-wk experiment.

The amount of feed provided was equal to that needed to meet energy requirements based on size of cow, age and production during the preliminary

period. The concentrate to forage ratio was adjusted for the entire group of cows at the end of each period based on the expected decrease in milk production. Cows received their completely mixed ration in individual stalls twice daily. Enough feed was offered to each cow so that some orts accumulated every day. When orts exceeded 10% of the feed offered on 2 or 3 days during a given week for any cow, the amount of feed presented to the cow was reduced.

Orts were collected and refrigerated daily and composited weekly. Composite feed concentrate mixtures, sorghum silage and prairie hay and orts samples were dried to a constant weight at 40C, ground in a Wiley mill (2 mm screen) and sampled for laboratory analyses. Laboratory analyses included dry matter and total protein (A.O.A.C., 1975). Milk yields of individual cows were recorded twice daily. Milk samples were collected at four consecutive milkings each week for milk fat and total protein analysis. Milk fat was determined with a Mark II Milk-O-Tester (A/S N. Foss Electric, Denmark) and milk protein percentage was determined during the fourth week of each period by the Kjeldahl method (A.O.A.C., 1975). Cows were weighed on 3 consecutive days at the end of the preliminary adjustment period and at the end of each period of the trial.

Results and Discussion

In Situ Experiment

Laboratory analysis failed to detect a quantitatively important difference between CSBM and HSBM in nitrogen (N) solubility. Nitrogen solubility in .15 M NaCl (Waldo et al., 1979) was 11.2% of total N in the HSBM versus with 19.3% for the CSBM meal. Though these differences were larger than in a previous study (Chapter IV), they were not as large as differences reported by Netemeyer et al. (1982), i.e., 8.1 versus 24.6%. The difference between CSBM and HSBM in *in situ* disappearance of dry matter (DM) and nitrogen also were not significant (Table

5). Heat treatment tended to increase rates of DM and N disappearance. As in previous work reported in Chapter IV, HSBM had a higher rate of N disappearance than CSBM.

Lactation Trial

Voluntary dry matter intakes by cows were slightly but not significantly greater for cows fed HSBM at both production levels (Table 6). Total protein intake was slightly above NRC (1978) proposed protein requirements for cows at high production levels and slightly below proposed requirements for lower producing cows (Table 4). Netemeyer et al. (1982) observed a positive response to feeding heat treated soybean meal when protein intake exceeded NRC (1978) requirements by 30%. But in a continuous feeding trial by Grummer and Clark (1982), milk yields of cows were not altered by nitrogen solubility of soybean meal flakes.

Gross efficiency of feed utilization for milk production (milk yield per unit of dry matter consumed) differed with milk production level (Table 6) tending to be improved by HSBM only with the higher production group. Total milk yield for the entire experiment was slightly higher for the cows in the high group fed HSBM than for cows fed CSBM. For cows in the lower production group, daily milk yield of cows initially assigned to receive HSBM was higher than that of cows fed CSBM (Figure 1) and no benefit was apparent from the heat treatment of soybean meal. For the higher production group, milk yield of cows fed both types of SBM were similar at the start of the trial and yield tended to be higher from cows fed HSBM (Figure 2). In nine of the ten pairs in the high production group, production was greater from HSBM than CSBM though the overall treatment means were not significantly different. Milk composition was not affected by treatment (Table 6). Weight change was positive but similar for cows fed both meal types

reflecting that energy intake by cows in all groups was adequate.

In conclusion, heat treatment of SBM tended to increase feed intake and milk yield without changing milk composition of high producing cows even though heat treatment failed to reduce degradation rate in situ. Reasons for lack of benefit at lower production levels remain to be determined. Perhaps protein requirements specified by NRC (1978) are excessive for low-producing cows but inadequate for high producing cows. At lower protein levels, increasing protein escape could be deleterious if adequate levels N are not provided for microbial protein synthesis. Specific procedures to manufacture and pre-test a heat-treated soybean meal for high producing dairy cows need to be established.

Table 1. INGREDIENT COMPOSITION AND PROTEIN CONTENT OF DIET DRY MATTER FOR COW USED FOR IN SITU DACRON BAG EXPERIMENT[a]

Item	IRN[b]	%
Ingredient		
Corn, ground	4-21-018	30.0
Soybean meal	5-04-604	13.5
Sorghum, grain	4-04-383	10.5
Molasses, cane	4-04-696	4.5
Dicalcium phosphate	6-01-080	1.2
Salt, trace mineral[c]		.3
Sorghum silage	3-04-468	28.0
Prairie hay	1-03-191	12.0
Protein, % of CM		13.6

[a]Contained 13.6% protein.

[b]International reference number.

[c]Morton Salt Co., Chicago, IL 60606; contained as a percentage: NaCl not more than 97%, not less than 92%.

Table 2[a]. INGREDIENT COMPOSITION AND CHARACTERISTICS OF CONCENTRATE DRY MATTER[a]

Item	IRN[b]	Protein Group	
		High	Low
Ingredient, %			
Corn, ground	4-21-018	66	72
Soybean meal	5-04-604	26	20
Molasses, cane	4-04-696	5	5
Dicalcium phosphate	6-01-080	1	1
Limestone	6-02-632	1	1
Salt, trace mineral[c]		1	1
Calculated composition crude protein, %		17.8	16.0
Net energy (NE_{ℓ}), Mcal/kg		1.82	1.83

[a]Dry Matter Basis.

[b]International reference number.

[c]Morton Salt Co., Chicago, IL 60606; contained as a percentage: NaCl not more than 97%, not less than 92%.

Table 3. INGREDIENT COMPOSITION AND CHARACTERISTICS OF DIET DRY MATTER[a]

Item	IRN[b]	Protein Level			
		Low		High	
		PDI-40	PDI-10	PDI-40	PDI-10
Ingredient, %					
Corn, ground	4-21-018	43.2	43.2	39.6	39.6
Soybean meal (PDI-40)	5-04-604	12.0	--	15.6	--
Soybean meal (PDI-10)	5-04-604	--	12.0	--	15.6
Molasses, liquid	4-04-696	3.0	3.0	3.0	3.0
Dicalcium phosphate	6-01-080	.6	.6	.6	.6
Limestone	6-02-632	.6	.6	.6	.6
Salt, trace mineral[c]		.6	.6	.6	.6
Sorghum silage	3-04-468	28.0	28.0	28.0	28.0
Prairie hay	1-03-191	12	12	12	12
Measured protein, % DM		14.3	14.6	16.8	17.0
Solubility of SBM-N in .15M NaCl		19.3	11.2	19.3	11.2
NE_ℓ (Mcal/kg of DM), calculated		1.68	1.68	1.68	1.68

[a]Dry Matter Basis.

[b]International reference number.

[c]Morton Salt Co., Chicago, IL 60606; contained, as a percentage: NaCl not more than 97%, not less than 92%.

Table 4. PROTEIN INTAKE OF COWS RELATIVE TO NRC STANDARD

Item	Period of Experiment[a]			
	1	2	3	4
	%			
Low Group				
SBM PDI-40	75	91	97	85
SBM PDI-10	81	98	107	95
High Group				
SBM PDI-40	100	117	104	105
SBM PDI-10	105	115	108	102

[a]Each period represents 4 weeks of the trial.

Table 5. DISAPPEARANCE OF DRY MATTER (DM) AND NITROGEN (N) FROM SOYBEAN MEAL TYPES (SBM) PLACED IN DACRON BAGS AND SUSPENDED IN THE RUMEN

	Exposure Time, h	SBM Type		
		FSBM	HSBM	CSBM
DM disappearance, %[a]	0	29.2	24.8	23.9
	4	36.8	38.4	35.1
	12	48.7	54.2	48.3
	20	70.9	73.8	68.0
Residue slope,				
%/h[b]		- 2.1	- 2.2	- 2.1
%/h[c]		- 4.9	- 5.3	- 4.4
N disappearance, %[a]	0	6.0	7.3	8.6
	4	12.4	20.5	11.9
	12	19.5	32.4	24.5
	20	49.8	64.5	49.1
Residue slope,				
%/h[b]		- 2.3	- 2.8	- 2.3
%/h[c]		- 3.5	- 5.0	- 3.4

[a]Each value is an mean of two observations.

[b]Linear

[c]Logarithmic

Table 6. EFFECT OF HEATING SOYBEAN MEAL ON RESPONSES OF COWS

Item	Production Group				SE
	Low		High		
	CSBM	HSBM	CSBM	HSBM	
Number of cows	10	10	10	10	
Dry matter intake, kg/day	19.6	19.9	22.6	22.9	.33
Total protein intake, kg/day	2.8	2.9	3.8	3.9	.06
Protein intake, % of DM	14.3	14.6	16.8	17.0	--
Weight change, kg/day	.43	.44	.49	.51	.17
Milk Yield					
Milk, kg/day	25.9	25.1	31.0	33.5	.31
Fat, %	3.8	3.7	3.6	3.6	.06
Protein, %	3.10	3.17	2.96	2.98	.05
Milk efficiency	1.32	1.26	1.37	1.46	.01
FCM	25.1	24.0	29.1	31.5	.02

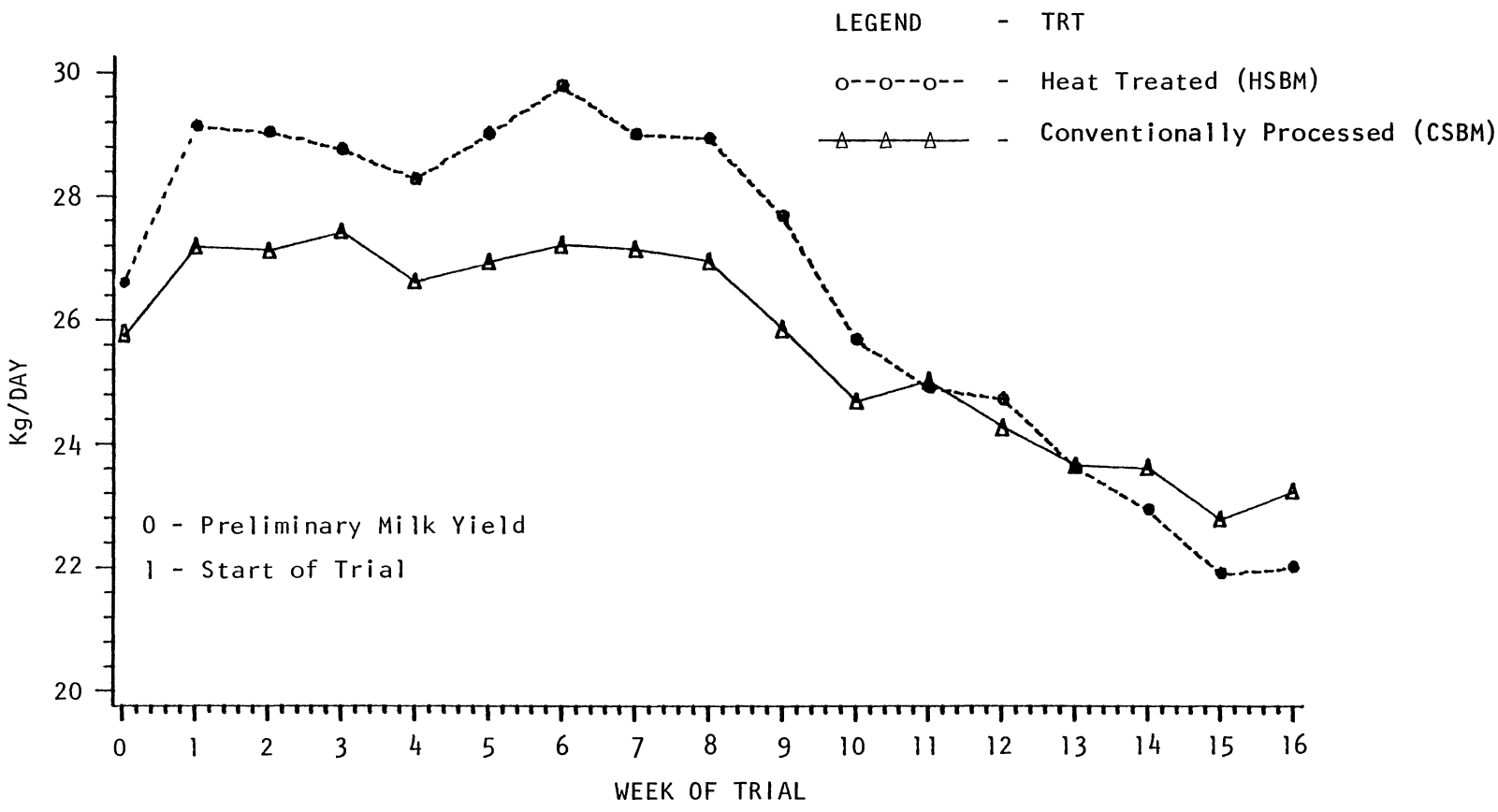

Figure 1. Milk Yield of Cows Fed Soybean Meals in Low Production Group

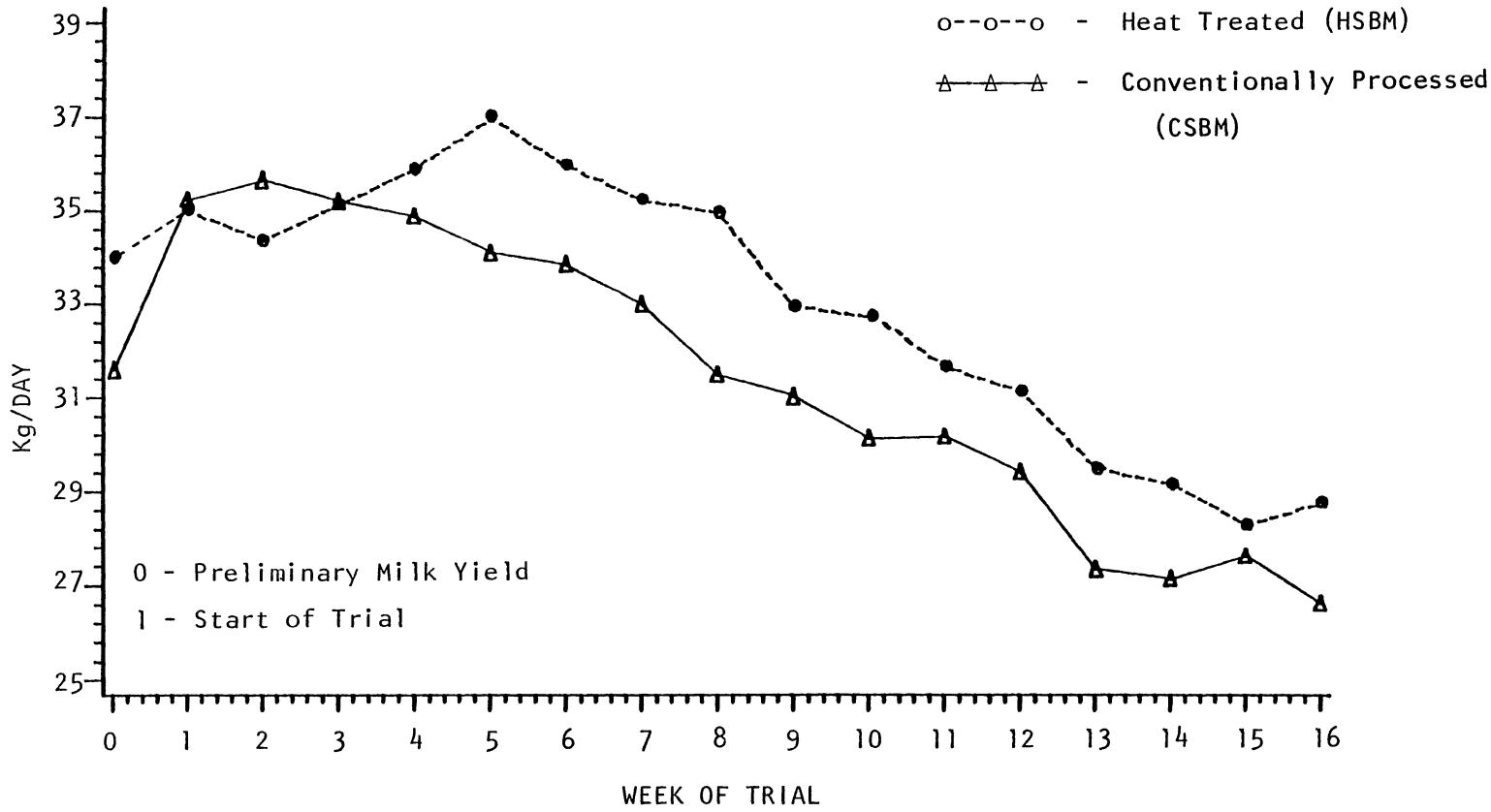

Figure 2. Milk Yield of Cows Fed Soybean Meals in High Production Group

CHAPTER VI

INFLUENCE OF HEAT TREATMENT OF SOYBEAN MEAL ON RUMINALNITROGEN ESCAPE AND UTILIZATION BY LACTATING DAIRY COWS

Summary

Five lactating dairy cows with duodenal T-type cannulas were fed diets containing either feed grade SBM (CSBM) or heat treated (HSBM) to estimate feed nitrogen bypassing ruminal degradation under typical production conditions in a replicated crossover experiment yielding 10 observations per mean. Diet dry matter consisted of 60% concentrate, 12% prairie hay and 28% sorghum silage formulated to provide 1.68 Mcal NE_l/kg. Nitrogen solubility of the soybean meals was 11.2 and 19.3% of total nitrogen and protein from SBM accounted for 61% of the total protein intake. Organic matter digestion coefficients were similar in the rumen (55.0 versus 52.5), post-ruminally (37.1 versus 37.6) and in the total tract (72.4 versus 71.5). Dry matter intakes, milk yield and chemical composition were similar between treatments and total N entering duodenum, microbial N and ammonia N were also similar. Feed nitrogen escaping ruminal degradation was slightly higher for HSBM than CSBM diet (58.1 versus 56.1%). The difference between diet duodenal feed nitrogen divided by amount of feed nitrogen for the CSBM diet calculates to equal 2.8% higher ruminal protein escape with heat treatment. Mean cow response with regard to dietary N was similar in the rumen (34.7 versus 31.8%), post-ruminally (62.7 versus 62.1%) and in the total tract (71.3 versus 69.8 for the CSBM and HSBM diets.). The extra heating of SBM used under

the production conditions of this study did not significantly increase ruminal escape of feed protein.

(Key Words: Soybean meal, Rumen, Nitrogen bypass, Processing.)

Introduction

Considerable interest has been shown in modifying proteins to increase their resistance to microbial attack and loss in the rumen (Chalupa, 1975; Huber et al., 1981; Santos et al., 1984). The value of dietary protein to ruminants is influenced largely by the proportion of the protein escaping ruminal degradation. Heat treatment may improve protein utilization in ruminants by increasing ruminal escape. Heat-treated proteins that are relatively resistant to ruminal degradation yet available in the small intestine are desirable for supplementation of young growing ruminants and high producing dairy cows (Sherrod and Tillman, 1962; Mielke and Schingoethe, 1981; Netemeyer et al., 1982).

Solubility was substantially decreased when plant proteins were extensively heated (Glimp et al., 1967; Nishimuta et al., 1972; Tagari et al., 1962). Protein degradation in the rumen also was reduced by heating (Yu, 1978; Broderick and Craig, 1980; Lindberg et al., 1982). Ruminal degradation of protein in seed meals can be reduced by supplying extra heat during pressure or solvent extraction of oil from the seed. Factors such as heating time, temperature and feed flow rate are important to optimize the conditions for ruminal protection. A poor combination of these factors may overprotect a protein so that it is neither fermented in the rumen nor digested in the small intestine.

Nutrient intake and body stores have a tremendous influence on peak lactation and persistency of lactation in dairy cows. Broster and Strickland (1977) demonstrated that with every kilogram increase in peak milk production, milk yield during the entire lactation period increased by 200 kg. Protein often-times

becomes a limiting nutrient in early lactation, especially with high producing cows. Increasing total protein percentage of the diet in early lactation, has not always increased milk production. The amount and type of protein, genetic potential of cows and management factors could certainly affect response (Foldager and Huber, 1979).

The objective of our study was to measure ruminal escape of feed nitrogen in diets containing commercially prepared regular and more extensively heated soybean meal.

Materials and Methods

Soybean meals prepared by the flash desolventizing system and classified according to protein dispersibility index (PDI) were obtained from a commercial manufacturer[1]. PDI is a measurement of the degree of heat treatment as described by A.O.C.S. (1969). The lower the PDI value the greater the heat treatment. More extensively heat treated soybean meal (HSBM) with a PDI of 10 and regular soybean meal (CSBM) with a PDI of 40 were fed in a ruminal nitrogen escape study to measure the amount of feed nitrogen that escaped degradation in the rumen. Diets were formulated to provide 1.68 Mcal net energy for lactation (NE_l) per kg of dry matter (Table 1). Soybean meals (SBM) comprised 31% of the total concentrate mixture used in the study (Table 2) and protein from SBM accounted for 61 per cent of the total protein intake. Diets were formulated to meet National Research Council (NRC, 1978) feeding standards. Diet dry matter consisted of 60% grain mix, 28% sorghum silage and 12% prairie hay.

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[1]Farmland Industries, St. Louis, MO.

Prior to initiation of the study, five mature cows (four Holstein and one Ayrshire) approximately 546 kg were fitted with T-type cannulas in the duodenum proximal to the bile duct. These lactating cows were used to estimate the amount of feed nitrogen escaping ruminal degradation under typical production parameters. Post-calving, cows were adjusted to diets with a 60:40 concentrate to forage ratio and were continued on diets with this percentage of concentrates throughout the study from week 4 to 22 of lactation.

In a replicated crossover design as described by Anderson and McLean (1974), diets with chromic oxide (.2% of DM) as the indigestible marker were fed in equal portions every 8 hours. Cows were fed in individual stalls three times daily (0300, 1100, 1900 hours) and feed weights were recorded daily. Cows were fed diets 2 wks prior to each two 4-day sampling period. Duodenal and fecal samples were obtained at 0300, 1100 and 1900 hours on day 4 through day 7 of each sampling period. To obtain fecal samples, cows were induced to defecate and the last portion of feces excreted was taken as the sample. On day 4 through 7, approximately 250 ml of duodenal digesta and 250 g wet feces were collected. Samples from each cow within each sampling period were composited on an equal wet basis and dried for 48 hours in a 60C forced air oven. Feed samples were obtained daily for each sampling period and composited within each animal and period. All samples were ground in a Wiley Mill fitted with a 2 mm screen and stored for future analysis.

During each period, 1500 to 2000 ml of strained rumen fluid was collected in an iced flask for bacterial isolation from a rumen cannulated cow fed each experimental diet for 2 wks prior to sampling. Rumen fluid was strained through 6 layers of cheesecloth, centrifuged at 200 g for 5 min. to remove feed particles and protozoa. The supernatant fluid was centrifuged at 30,000 g for 15 min to precipitate bacteria. The pellet was washed twice, once with .9% saline solution

and once with deionized distilled water and recentrifuged at 30,000 g for 15 min after each washing. The pellet was then lyophilized and stored for later nucleic acid analysis.

Each day 10% of the feed weighback was collected and refrigerated during each sampling period. At the end of each period, feed weighbacks were composited and sampled. Composite feed and orts samples were dried to a constant weight at 40C, ground in a Wiley Mill (2 mm screen) and stored for laboratory analyses. Samples of the concentrate mixtures, sorghum silage and sudangrass hay also were collected each week and analyzed for dry matter and total protein. Milk yields were recorded twice daily at 0700 and 1900 hours. Milk samples were collected for four consecutive milkings each week, composited by volume and analyzed for milk fat and total protein. A Mark II Milk-O-Tester (A/S N. Foss Electric, Denmark) was used to measure milk fat. Milk protein percentages were determined during the experimental collection week by the Kjeldahl method (A.O.A.C., 1975).

Feed, duodenal and fecal samples were analyzed for dry matter (DM; 100C for 24 hr), ash (600C for 12 hr), Kjeldahl nitrogen (N; A.O.A.C., 1975) and chromium (Fenton and Fenton, 1979). Ammonia N was measured on the dried, ground duodenal samples by distilling the sample over magnesium oxide in a macro-Kjeldahl flask (A.O.A.C., 1975). Lyophilized bacteria and dried duodenal samples were analyzed for nucleic acid-N by the procedure of Zinn and Owens (1982). Nucleic acid-N was used as a microbial marker. Daily amounts of non-microbial DM and N flowing past the duodenal cannula were calculated by subtracting the microbial contribution from the total. Duodenal organic matter (DOM) flow, corrected for the microbial contribution, was calculated from the corrected duodenal DM flow times duodenal organic matter percentage. Data were analyzed by the general linear models prodcedure of the Statistical Analysis

System (SAS, 1979), considering soybean meal, cow and period as main effects.

Results and Discussion

Dry matter and crude protein intakes and body weight changes are presented in Table 3. Dry matter intake averaged 20.2 to 20.4 kg/day and was not significantly affected by diet. Since treatments were identically formulated (Table 1) no differences in crude protein intakes were expected. Body weight gain tended to be somewhat higher on the heat-treated diet. Average daily milk yield, milk fat, and milk protein for the treatments ranged from 24.9 to 25.0 kg., 3.16 to 3.15% and 3.16 to 3.16%, respectively (Table 4). Production of FCM was almost identical for cows fed with the two diets.

Ruminal organic matter digestion, both unadjusted and adjusted for microbial OM contributions, for the two diets was similar (Table 5). The proportion of organic matter digestion occurring in the rumen was slightly lower for the heat treated diet than the regular soybean meal diet (74.5 vs. 76.3%). Extents of post-ruminal digestion and total tract organic matter digestion were similar (37.1 vs. 37.6% and 72.4 vs. 71.5%, respectively). Apparent and true OM digestibilities indicated that diets were similar in ruminal fermentability as expected.

Nitrogen intake, flows and digestibilities were determined with chromic oxide as the indigestible flow marker. Amounts of total N microbial N and ammonia entering the duodenum were similar for both treatments (Table 6). Feed nitrogen escaping ruminal degradation was slightly higher for HSBM than the RSBM diet (336 vs. 327 g/day, respectively). Assuming other diet ingredients were equally degraded in the two diets, SBM N escape was increased by 2.8 percentage units by heat treatment. This change is very small. Percentage of nitrogen digested in the total tract, was slightly lower with HSBM than for CSBM, but

generally similar to those of Crickenberger et al. (1979), Sachteleben (1980) and Kung et al. (1983).

Microbial efficiency, g of microbial N/kg OM truly digested in the rumen, was 13.5 and 14.1 for the two groups, and was not affected by soybean meal treatment. Bacterial efficiencies in this study were lower than average values reported in a review by Stern and Hoover (1979) of 27g of microbial N synthesized per kg of OM truly digested in the rumen, though reported values ranged from 10 to 49 g. Goetsch and Owens (1985a) reported mean values of 19.0 to 29.8 g microbial nitrogen/kg organic matter truly fermented in the rumen.

Digestibilities of N in the total tract were compared to the calculated digestible protein from the standard relationship (percent digestible protein = .9 x percent crude protein-3; NRC, 1976) presented in Table 6. Calculated values were extremely close to measured total tract N digestibilities.

None of the nitrogen and organic matter parameters measured were significantly altered by heat treatment of SBM. The percentage of ruminal escape of total feed nitrogen was only slightly higher in cows fed extra heat SBM compared to the control group (58.1 vs. 56.1%). Extra heating of soybean meal appeared to be ineffective in protecting soybean meal from ruminal degradation. Availability of the N in the small intestine was not measured, but post-ruminal digestion was not reduced. Plegge et al. (1982) suggested that roasting decreased ruminal degradation of soybean meal protein but did not significantly reduce post-ruminal availability. Ruminal escape estimates for soybean meal from in vivo experiments have been quite variable ranging from 15 to 61% (Hume, 1974; Zinn et al., 1981; Loerch et al., 1983b). Most estimates have used surgically altered animals fed restricted amounts of feed under steady state conditions; thus, the preceding means may not be applicable to lactating dairy cows. Ruminal pH will influence solubility and degradability of soybean meal in the rumen (Isaacs and

Owens, 1972; Wohlt et al., 1973; Loerch et al., 1983a). High levels of corn grain may reduce pH and increase the amount of regular soybean meal protein escaping ruminal degradation and may have redued the response to heat treated SBM in this experiment.

Based on these data and those of a previous continuous feeding trial, it appears that the heat-treated soybean meal obtained from Farmland Industries, St. Louis, MO, for this particular trial was not sufficiently treated to increase the amount of feed protein escaping ruminal degradation. Nevertheless, the values obtained on the percentage of feed protein escaping degradation in the rumen of lactating cows consuming a 60% concentrate ration at the levels achieved in this trial are of interest. This study provided useful methods and reference points for future trials in which the extent of degradation of different sources of feed protein were estimated.

Table 1. INGREDIENT COMPOSITION AND CHARACTERISTICS OF DIET DRY MATTER

Item	IRN[a]	Treatment	
		CSBM	HSBM
Ingredients, %			
Corn, ground	4-21-018	36.57	36.57
Soybean meal (PDI-40)	5-04-604	19.2	0
Soybean meal (PDI-10)	5-04-604	0	19.2
Molasses, cane	4-04-696	3.0	3.0
Dicalcium phosphate	6-01-080	.6	.6
Limestone	6-02-632	.6	.6
Salt, trace mineral[b]	---	.03	.03
Sorghum silage	3-04-468	28.0	28.0
Prairie hay	1-03-185	12.0	12.0
Protein, % of DM		17.6	17.6
Solubility of SBM-N in .15 M NaCl		19.3	11.2
NE_{ℓ} (Mcal/kg of DM), calculated		1.68	1.68

[a]International reference number.

[b]Morton Salt Co., Chicago, IL 60606; contained, as a percentage; NaCl not more than 97%, not less than 92%.

Table 2. INGREDIENT COMPOSITION AND CRUDE PROTEIN CONTENT OF CONCENTRATE DIETS[a]

Item	IRN[b]	Treatment	
		CSBM	HSBM
Ingredient, %			
Corn, ground	4-21-018	61.05	61.05
Soybean meal PDI-40	5-04-604	31.45	---
Soybean meal PDI-10	5-04-604	---	31.45
Molasses, cane	4-04-696	5.0	5.0
Dicalcium phosphate	4-01-080	1.0	1.0
Limestone	6-02-632	1.0	1.0
Salt, trace mineral[c]	---	.05	.05
Crude protein, %		23.4	23.2

[a]Dry Matter Basis.

[b]International reference number.

[c]Morton Salt Co., Chicago, IL 60606; contained, as a percentage; NaCl not more than 97%, not less than 92%.

Table 3. RESPONSES OF COWS FED REGULAR AND HEAT TREATED SOYBEAN MEAL

Item	Treatment		
	CSBM	HSBM	SE
Number of observations	10	10	
Feed Intake			
Dry matter, kg/day	20.2	20.4	.23
Total protein, kg/day	3.6	3.6	.05
Protein, % of DM	17.8	17.6	
Weight change, kg/day	0.7	1.6	.63

Table 4. YIELD AND COMPOSITION OF MILK OF COWS

Item	Treatment		SE
	CSBM	HSBM	
Milk, kg/day	24.9	25.0	.81
Fat, %	3.16	3.16	.04
Protein, %	3.16	3.16	.04
FCM[a], kg/day	21.4	21.5	.39
Milk/feed DM	1.23	1.23	---

[a]FCM = 0.4 (kg milk/day) + 15 (kg fat/day).

Table 5. ORGANIC MATTER DIGESTION IN COWS FED REGULAR AND HEAT TREATED SOYBEAN MEAL

Item	Treatment		SE
	CSBM	HSBM	
Intake, g/d	18784	18966	
Leaving abomasum, g/d			
Total	8405	8832	280.7
Non-microbial	7243	7643	244.2
Ruminal digestion, %			
Unadjusted	55.0	53.3	1.2
Adjusted	55.0	52.5	1.2
Ruminal digestion			
% of total	76.3	74.5	1.6
Feces, g/d	5188	5337	101.9
Post-ruminal digestion % of input	37.1	37.6	1.7
Total tract digestion, %	72.4	71.5	0.5

Table 6. NITROGEN (N) DIGESTION IN COWS FED REGULAR AND HEAT TREATED SOYBEAN MEAL

Item	Treatment		SE	P
	CSBM	HSBM		
Intake, g/d				
Total	578	577		
SBM-N	353	352		
Leaving abomasum, g/d				
Total N	453.2	467.6	14.5	.62
Microbial N	99.8	102.9	3.6	.67
Ammonia	26.4	28.7	0.9	.24
Non-ammonia, non-microbial	327.0	336.0		
Feed N Bypass, %	56.1	58.1	1.48	.68
Ruminal digestion, %				
Unadjusted	21.8	18.7	1.9	.42
Adjusted[a]	34.7	31.8	1.7	.40
Microbial efficiency, g				
Microbial N/kg OM truly digested in rumen	14.1	13.5	.3	.42
Ruminal digestion, % of total	30.2	26.7	2.6	.50
Feces, g/d	166.8	172.9	3.8	.43
Post-ruminal digestion, % of input	62.7	62.1	6.3	.75

Table 6 (Continued)

Item	Treatment		SE	P
	CSBM	HSBM		
Total tract digestion, %	71.3	69.8	0.5	.13
Expected total digestion[b], %	73.2	73.0		

[a]Adjusted for microbial and ammonia nitrogen.

[b]Calculated as % digestible protein = [.9 (% crude protein) -3]/% crude protein.

CHAPTER VII

SOYBEAN MEAL N RUMINAL ESCAPE, TURNOVER RATE AND IN SITU DISAPPEARANCE IN LACTATING COWS FED DIETS AT TWO CONCENTRATE TO FORAGE RATIOS

Summary

Diets with two concentrate to forage ratios, with or without feed grade soybean meal (SBM) were fed to six lactating dairy cows with duodenal T-type cannulas in a 2 x 2 factorial design. SBM labeled with Yb was used to predict SBM passage rate. Using the in situ dacron bag procedure, dry matter (DMD) and nitrogen disappearance (ND) rates were measured in the rumen of a cannulated cow. Diets consisted of concentrate with SBM or urea, sorghum silage and alfalfa hay with concentrate at 65 and 35% of diet dry matter. Dry matter intake ranged from 16.2 to 18.4 kg/day while milk yield ranged from 19.3 to 23.1 kg. Of the SBM nitrogen fed, 46 and 18% escaped ruminal digestion with 65 and 35% concentrate diets. Post-ruminal N digestion (% of input) ranged from 62 to 75%. Rumen turnover rates for SBM were 8.7 and 8.3%/h for 65 and 35% concentrate diets. Logarithmically estimated degradation rates of SBM nitrogen were 5.3 and 10.7 for the 65 and 35% concentrate diets, respectively while N solubility in saline was 9.8%. Predicted SBM nitrogen escape, base on these passage and degradation rates and solubilities were 56.1 and 39.3 for the 65 and 35% concentrate diets, respectively.

(Keywords: Soybean meal, Nitrogen, Escape, Ruminants.)

Introduction

Estimates from *in vivo* trials of soybean meal (SBM) nitrogen escaping ruminal degradation range from 10 to 61% (NRC, 1985). Some of this variation is due to differences in choice of inherent and microbial markers as well as to the method of calculation. Escape can be estimated by regression or by comparison with a basal diet which may or may not be isocaloric and isonitrogenous with the test diet. Application of a single escape estimate to formulate diets for dairy cattle remains tenuous due to this variation. Further, extent of protein degradation in the rumen is not a constant but has been shown to vary with dietary factors (dry matter intake and feed particle size) which in turn alter ruminal pH and passage rate (Weakley, 1983). Estimates of the amount of SBM nitrogen escaping ruminal degradation with one exception were derived from trials with steers or lambs. The only dairy cow trial by Goetsch and Owens (1985) estimated 35% ruminal protein escape of SBM but was based on an assumed escape value for corn grain.

The two factors which dictate how much protein escapes degradation are residence time of feed protein in the reticulo-rumen and rate of proteolysis. Proteolytic rate can be estimated by *in situ* procedures, but retention time of soybean meal in the rumen of lactating cows needs to be determined *in vivo* to estimate protein escape. Passage rates of SBM were estimated at 8.4%/h (Erdman et al., 1984) in dairy cows fed a 50% concentrate diet, and 3.2%/h and 4.1%/h (Teeter, 1981) in steers fed a 84% concentrate and 10% concentrate diet. *In situ* estimates of escape have proven useful previously to predict escape (Zinn and Owens, 1983). Escape values ($E = k_p/(k_p + k_d)$ were calculated by combining rates of passage (k_p) and digestion (k_d) with and without substraction for soluble N.

The objectives of this study were to directly estimate the fraction of soybean meal protein which escaped ruminal degradation and compare this with estimates based on in situ digestion and ruminal turnover rates.

Materials and Methods

Two months prior to calving, six dairy cows were surgically prepared with T-type cannulae in the doudenum proximal to the pancreatic and bile ducts. Cows were assigned to four treatments in a 2 x 2 factorial design (Snedecor et al., 1980). Experimental diets consisted of two concentrate to forage ratios; 65:35 and 35:65. Periods lasted 2 wk each with the first 9 d for adaption to diets with data collection the last 5 d. Twelve days were allocated for adaptation to diets when the concentrate to forage ratio was changed.

Diet ingredients consisted of a concentrate mixture, alfalfa hay and sorghum silage (Table 1). Ingredients were selected to typify those ingredients used under practical management conditions. The concentrate mixture consisted of ground corn plus either soybean meal that had been processed in a conventional manner or urea plus corn starch (Table 2). Of the total N in SBM, 9.8% was soluble in .15N saline solution. Diets were formulated so that the percentage of corn remained constant between test diets. Formulation of diets in this manner permitted soybean meal escaping ruminally degradation to be estimated by difference.

Concentrate, alfalfa hay and sorghum silage were fed to cows as a complete mixture at 0400 and 1800 hours each day. Chromic oxide was added to the concentrate portion of the diet at levels to be equal to .2% on a dry matter basis of the total mixed ration to provide a digesta flow marker. Indigestible neutral detergent fiber (INDF) was used as a marker to calculate duodenal digesta flow of organic matter and nitrogen (Goering and Van Soest, 1970). Each day, 10% of the feed weighback was collected and refrigerated. Composite feed and orts samples

were dried to a constant weight at 40C, ground in a Wiley mill (2 mm screen) and stored for laboratory analyses. Samples of the concentrate, sorghum silage and alfalfa hay also were collected each week and analyzed for dry matter and total protein. Milk yields were recorded twice daily at 0600 and 1800 hours. Milk samples were collected for four consecutive milkings each week, composited by volume and analyzed for milk fat and total protein. Milk fat content was determined using a Mark II Milk-O-Test (A/S N. Foss Electric, Denmark). Milk protein percentages were determined during each collection period by the Kjeldahl method (A.O.A.C., 1975).

Duodenal and fecal samples were collected at 0600, 1000 and 1400 hours from each cow for three and five consecutive days, respectively. Approximately 250 ml of duodenal digesta and 250 g wet feces were collected each day. To obtain fecal samples, cows were induced to defecate and the last portion of the feces excreted was taken as the sample. Samples from each cow within each sampling period were composited on an equal wet basis and dried for 48 h in a 60C forced air oven. Feed, duodenal and fecal samples were analyzed for dry matter (DM; 100C for 24 h), ash (600C for 12 h), Kjeldahl nitrogen (N; A.O.A.C., 1975) and chromium (Fenton and Fenton, 1979). Ammonia N was measured on the dried, ground duodenal samples by distillation over magnesium oxide in a macro-Kjeldahl flask (A.O.A.C., 1975).

A rumen fistulated cow was randomly assigned to a treatment sequence of the diets used in the experiment. During each period, 1500 to 2000 ml of strained rumen fluid was collected for isolation of bacteria. Rumen fluid was centrifuged at 200 g for 5 min. to remove feed particles and protozoa. The supernant fluid was centrifuged at 30,000 g for 15 min. to precipitate bacteria. The pellet was washed once with .9% saline solution and once with deionized distilled water and recentrifuged at 30,000 g for 15 min. after each washing. The pellet was then

lyophilized and stored for analysis of total N and for nucleic acid-N by the procedure of Zinn and Owens (1982).

On day two of each collection period, the rumen cannulated cow was used to evaluate in situ digestibility of individual diet components (alfalfa hay, sorghum silage and SBM) using a dacron bag procedure. Substrate samples weighing approximately 1 g were placed in 8 x 12 cm bags with pores between 50 to 75 microns in diameter. Bags were constructed from dacron cloth (100% polyester, R102 Marvelaire White; Erlanger, Blumgart and Co., Inc., 1450 Broadway, New York, New York 10018) using a single piece of material measuring approximately 8 x 24 cm that was folded in half and double sewn along two of the open edges with polyester thread. The top was left open for sample insertion into the bag.

Water-proof glue (Duco cement, DuPont Co., Wilmington, DE 19898) was applied to the stitched area to prevent loss of small particles through the needle holes. All cut edges were singed with a hot knife to prevent fraying in the rumen. The constructed bags had a surface area of approximately 192 cm^2. Prior to filling, bags were machine washed and dried for 24 h at 100C, cooled in a dessicator and weighed. After addition of samples, bags were tied with nylon yarn. Bags were individually tied to the end of a 60 cm yarn line and grouped by time. Individual bags were labeled with a waterproof marker. Approximately 5 cm from the end of the line, a 30 g steel nut was tied to weight the bags down.

Sample bags in duplicate were incubated in the ventral sac of the rumen. Bags were introduced at the same time and withdrawn at respective intervals to standarize time after feeding effects. Bags were washed under a stream of tap water (approximately 26C) until the rinsing water was clear. Washing time averaged approximately 150 sec/bag. Bags were dried in a forced air oven for 48 h at 60C, cooled in a dessicator and weighed. Bag contents were analyzed for total N by Kjeldahl procedure (A.O.A.C., 1975).

On day 5 of each collection period, 250 g of soybean meal labeled with ytterbium as described by Teeter (1982) were fed to each intestinally cannulated cow. Starting 12 h post feeding, approximately 400 ml of duodenal digesta was collected every 12 h for 2 d. Duodenal samples were analyzed for Yb according to Ellis (1982). On day 7 of the collection period, approximately 250 mls of rumen fluid was collected by stomach tube from each intestinally cannulated cow. Samples were acidified with sulfuric acid and analyzed for ammonia (A.O.A.C., 1975).

Results and Discussion

Laboratory and In Situ Analyses

In situ nitrogen disappearance (ND) for soybean meal, alfalfa hay and sorghum silage (Table 3) tended to be higher when the low concentrate diet was fed (DM and ND plots of feed substrates are presented in Appendix B). Protein content and diet composition presumably altered the ruminal flora or fauna enough to alter rate of disappearance. Generally, in situ disappearance of plant proteins is greater with roughage than with concentrate diets (Weakley, 1983).

Alfalfa hay N and DMD were variable across experimental diets (Table 3). Miller (1982) and Barrio (1984) reported similar values for DM and N disappearance at 24 h. Disappearance of N and DM from soybean silage were lower with the 65% concentrate than the 35% concentrate diet. In contrast, Barrio (1984) reported lower ND and DMD of sorghum silage with a 60% concentrate diet than for a 80% concentrate diet. Analytical problems associated with microbial attachment to feed complicated estimation of ND at 24 h. Why the sorghum silage ND and DMD were higher with the 35% than the concentrate diet are presumably not due to pH since ruminal pH in this study was similar for the two diets (6.8 versus 7.0).

Ruminal passage rates for soybean meal labeled with ytterbium ranged from 7.9 to 8.7%/h. This is higher than the 4.9%/h reported by Stern et al. (1983) for lactating dairy cows ad libitum fed a basal diet of 60% alfalfa hay and 40% concentrate (Table 4) but similar to an estimate of 8.4%/h from Erdman et al. (1984). They fed lactating cows diets containing 30% corn silage, 10% alfalfa haylage, 10% alfalfa hay and 50% concentrate (DM basis). The SBM ruminal passage rate in the present trial tended to be higher with the high concentrate diet than with low concentrate diet, means were not statistically different ($P > .95$).

Rates of ND from soybean meal in situ tended to be higher if SBM was included in the diet. Loerch et al. (1983) also reported that ND from SBM in situ was greater with SBM in the diet. This may reflect microbial adaption to degrade SBM or overall changes in microbial activity in the rumen with SBM in the diet.

One can predict ruminal escape of N from SBM using the equation

$$E = k_p/(k_p + k_d)$$

where k_p and k_d are fractional passage and digestion rates. Using values from Tables 3 and 4 as applied to total N in SBM, predicted escapes values are 62 and 44%. If soluble N of SBM is deducted prior to calculations, predicted escape values are 56 and 39%, respectively.

In Vivo Trial

Mean dry matter (DM) intake ranged from 16.2 to 18.4 kg/day for the treatment groups (Table 5). DM intake was highest ($P < .01$) for cows receiving the low concentrate level with urea supplementation. DM intake may have been higher to compensate for the lower crude protein content and caloric density of

the diet. Body weight gain tended to be greater with the high concentrate diets. Ruminal ammonia levels tended to be higher with the lower concentrate diets and with soybean meal in the diet. Added corn starch may have increased ammonia use by ruminal microbes and decreased ammonia concentrations in the rumen.

Mean daily milk yield, milk fat and milk protein for the experimental diets ranged from 19.3 to 23.1 kg, 3.7 to 3.8% and 3.4 to 3.8%, respectively (Table 6). Mean milk yield tended to be higher in the high concentrate diets reflecting the higher protein and caloric density of these diets versus the low concentrate diets. FCM production was higher for cows fed the 65% than for those fed the 35% concentrate diet ($P > .02$).

Duodenal flow of OM was variable across diets ($P > .37$) and non-microbial N flow reflected treatment OM intakes (Table 7). Post-ruminal digestion as a percentage of duodenal flow was greater with the 65% concentrate diets ($P < .01$). Total tract OM digestion using chromic oxide as the digesta flow marker, tended to be higher for the high concentrate diets. Yet, total tract digestibilities were lower than anticipated for these diets and tended to be lower than values reported by Goetsch and Owens (1985).

Ruminal digestion of OM averaged 48%, higher than many values reported in previous studies. OM digestion in the stomach as a percentage of OM of intake in a study by Stern et al. (1985) was 30 $\pm$ 4% and similar digestibilities were reported by Santos et al. (1984) with lactating cows. Kung et al. (1983) observed very low estimates of OM digestion (OMD) in the rumen (4 to 26% of OM intake) using La as an indigestible marker. When acid detergent lignin flow was used to calculate ruminal OMD, estimates ranged from 36 to 43% of OM intake. Kung et al. (1983) reported true OMD in the reticulo-rumen based on La was 27 $\pm$ 4 and based on acid detergent lignin as a marker was 48%.

In most studies with intestinally-cannulated cows, daily dry matter intakes reported have been restricted 6 to 10 kg. Such values are low for lactating dairy cows. Santos et al. (1984) observed higher daily intakes (14 to 16 kg). Sutton and Oldham (1977) and Hvelplund et al. (1976) concluded that as feed intake increases, the flow of digesta through the forestomachs increases which reduces the portion of OM digested in the rumen. When daily DM intakes increased from 8.2 to 12.9 kg in dairy cows fed hay and concentrates, OM disappearance in the rumen dropped by 4 percentage units (Tamminga et al., 1979).

Nitrogen duodenal flow and digestibility were calculated based on INDF as the digesta flow marker. Total N intakes reflected the differences in diet formulations (Table 8). To determine the amount of soybean meal escaping ruminal degradation, urea plus corn starch was substituted for soybean meal at each concentrate level. To help prevent urea toxicity, total N in the urea diet was slightly lower than in the SBM diets. Enough urea was added to the diets to maintain adequate N for microbial synthesis. Based on bacterial composition from rumen fluid of cows fed the experimental diets, N levels appeared to be adequate (Table 9). Both chemical composition and microbial efficiency (g N per kg OM truly digested in the rumen) were not significantly altered by diet ($P > .51$). Bacterial efficiencies were similar to those reported by Stern and Hoover (1979) and Goetsch and Owens (1985).

Non-ammonia non-microbial nitrogen passage to the duodenum tended to be greater with dietary SBM and with the 65 than the 35% concentrate diets $P>.21$). These reflect the difference in dietary protein escaping degradation in the rumen. Post-ruminal digestion (% of duodenal flow) tended to be higher with the 65% concentrate diets. Based on the differences in N flow leaving the abomasum between diets supplemented versus not supplemented with SBM, 45.9% and 18.0% of the SBM protein escaped ruminal degradation with the 65 and 35% concentrate

diets. These values are higher than those reported by Weakley (1983), i.e., 20.4 vs 13.8% for 80 and 40% concentrate diets, respectively. He assumed ruminal escape of corn protein to be 60% for calculation purposes, whereas in this trial, soybean protein ruminal escape was estimated directly since corn protein was constant within diets compared. Zinn and Owens (1983) observed SBM bypass values of 24 and 43% with 40 and 80% concentrate diets, again assuming corn protein escape value of 60%. In our study, increasing concentrate level from 35 to 65% increased ruminal escape of SBM nitrogen by 155%.

In a review by Schingoethe (1984), values of ruminal degradability of soybean meal were reported from 40 to 70%. Early investigations of Hume (1974) and Laughren (1978) reported ruminal escape values of soy protein of 61 and 32%, respectively. Though these estimates have been obtained in sheep studies and differences in terms of particle retention time in the rumen and chewing differences between sheep and cattle may limit the value of these data for extrapolation to cattle. Using regression techniques Stern and Satter (1982) estimated soybean meal protein escape in the rumen to be 35%. In other investigations using nitrogen disappearance data from dacron bags and a rate of pasage study, the estimate of soy protein escape was 38% on a 60% forage: 40% concentrate diet (Stern et al., 1983). The diet was not fed as a total mixed ration and .45 kg of soybean meal was fed at each feeding. The degradability estimates may be biased due to the method in which soybean meal was added which is different than under typical production conditions.

Most previous ruminal escape values for SBM have been confounded by other dietary protein sources. Though these values differ from other studies, these are more direct estimates of the amount of soybean meal protein escaping ruminal degradation at two concentrate to forage ratios than have previously been determined. Using nitrogen disappearance measurements from our dacron bag

study in conjunction with rate of passage data for soybean meal, SBM protein ruminal escape estimates were 62.2% for 65% concentrate and 43.7 for 35% concentrate diet versus 45.9 and 18% for *in vivo* estimates. Further research with lactating dairy cows with SBM and other high quality protein sources estimating ruminal escape are needed. Few estimates are available for high producing cows fed diets varying the forage to concentrate ratios and dry matter intakes. Using single constant values for ruminal escape of dietary soybean meal is imprecise. Adjustments for concentrate level and feed intake level need to be considered when using escape estimates in diet formulation.

Table 1. COMPOSITION OF DIET USED IN SBM NITROGEN RUMINAL ESCAPE EXPERIMENT

Item	IFN	Percent concentrate diet			
		65		35	
		1	2	1	2
		---------(%, dry matter)--------			
Ingredient					
Sorghum silage	3-04-468	17.5	17.5	32.5	32.5
Alfalfa hay, chopped	1-00-063	17.5	17.5	32.5	32.5
Concentrate (Table 2)		65	65	35.0	35.0
Crude protein		14.6	12.9	14.5	12.4

Table 2. COMPOSITION OF CONCENTRATE PORTION OF DIETS USED IN SBM NITROGEN RUMINAL ESCAPE EXPERIMENT

Item	IFN[a]	Percent concentrate in diet			
		65		35	
		1	2	1	2
Ingredient		---------(%, dry basis)---------			
Corn, ground	4-21-018	70.0	70.0	60.0	60.0
Soybean meal	5-04-604	18.0	--	28.0	--
Cornstarch	---	--	16.0	--	25.5
Cottonseed hulls	1-01-599	4.0	4.0	4.0	4.0
Urea	5-05-070	--	2.0	--	2.5
Molasses, cane	4-04-696	5.0	5.0	5.0	5.0
Dicalcium phosphate	6-01-080	2.0	2.0	2.0	2.0
Salt, trace mineral[b]		1.0	1.0	1.0	1.0
Chromic oxide		2.0	2.0	2.0	2.0
Crude protein		16.2	13.7	20.0	14.2

[a]International reference number.

[b]Morton Salt Co., Chicago, IL 60606; contained, as a percentage: NaCl - not more than 97.0%, NaCl - not less than 92.0%, Manganese - not less than .250%, Iron - not less than .200%, Sulfur - not less than .030%, Copper - not less than .033%, Cobalt - not less than .0025%, Iodine - not less than .007%, Zinc - not less than .005%.

Table 3. DISAPPEARANCE OF SOYBEAN MEAL, ALFALFA HAY OR SORGHUM SILAGE FROM DACRON BAGS IN RUMEN OF COW FED EXPERIMENTAL DIETS

	Exposure Time, hr	Percent concentrate in diet			
		65		35	
		1	2	1	2
		SBM			
DM disappearance, %	3	41.4	40.6	42.5	41.5
	6	46.9	46.4	44.3	47.0
	12	61.6	67.3	61.1	64.1
	24	85.2	83.5	80.1	93.3
Residue slope,					
%/h[a]		- 2.11	- 2.07	- 1.87	- 2.51
%/h[b]		- 6.70	- 6.28	- 5.27	-10.62
N disappearance, %	3	33.10	27.5	29.6	36.3
	6	43.6	33.9	50.0	37.4
	12	56.0	46.7	55.6	64.8
	24	78.3	62.5	92.9	80.8
Residue slope,					
%/h[a]		- 2.08	- 1.65	- 2.78	- 2.25
%/h[b]		- 5.29	- 3.15	-10.71	- 6.04
		Alfalfa hay			
DM disappearance, %	12	52.4	58.0	50.2	51.9
	24	64.4	71.1	65.5	65.5
	48	70.6	72.2	69.6	72.7
Residue slope,					
%/h[a]		- .47	- .34	- .49	- .54
%/h[b]		- 1.26	- 1.01	- 1.15	- 1.49

Table 3 (Continued)

	Exposure Time, hr	Percent concentrate in diet			
		65		35	
		1	2	1	2
		SBM			
N disappearance, %	12	66.5	67.0	66.3	80.67
	24	84.7	75.5	84.5	85.8
	48	84.5	76.6	88.0	89.4
Residue slope,					
%/h[a]		- .43	- .24	- .54	- .23
%/h[b]		- 1.83	- 2.07	- 2.61	- 1.33
		Sorghum silage			
DM disappearance, %	12	32.5	36.0	50.2	51.9
	24	38.2	39.3	65.5	65.5
Residue slope,	48	41.2	43.4	69.6	72.7
%/h[a]		- .23	- .20	- .49	- .54
%/h[b]		- .36	- .33	- 1.25	- 1.49
N disappearance, %	12	32.5	58.0	66.0	80.7
	24	42.4	71.1	84.5	85.8
	48	66.7	72.2	88.0	82.4
Residue slope,					
%/h[a]		- .96	- .34	- .54	- .58
%/h[b]		- 2.00	- 1.01	- 2.60	- 1.99

[a]Linear [b]Logarithmic

Table 4. RUMEN TURNOVER RATE OF Yb LABELED SBM IN COWS FED EXPERIMENTAL DIETS

	Percent concentrate in diet					
	65		35			
	1	2	1	2	SE	P
Rate, %/h	8.7	8.6	8.3	7.9	.4	.95

Table 5. RESPONSES OF COWS FED EXPERIMENTAL DIETS

Item	Percent concentrate in diet				SE	P
	65		35			
	1	2	1	2		
Dry matter intake, kg/day	16.6	16.2	16.7	18.4	.37	.01
Protein, % of DM	14.9	13.3	15.7	13.6		
Total protein intake, kg/day	2.47	2.16	2.62	2.51	.06	.01
Weight change, kg/day	.6	.8	.2	.1	.35	.83
Ruminal NH_3-N, mg/dl	8.6	5.7	11.7	7.8	1.2	.40

Table 6. MILK YIELD AND CHEMICAL COMPOSITION IN COWS FED DIETS AT TWO CONCENTRATE TO FORAGE RATIOS WITH OR WITHOUT SBM

	Percent concentrate in diets					
	65		35			
Item	1	2	1	2	SE	P
Milk, kg/day	23.1	21.0	19.8	19.3	.4	.61
Fat, %	3.7	3.7	3.7	3.8	.1	.94
Protein, %	3.5	3.4	3.7	3.7	.1	.38
FCM[a]	21.7	20.1	18.7	18.8	.6	.02

[a]FCM = 0.4 x kg milk/day + 15 x kg fat/day.

Table 7. ORGANIC MATTER DIGESTION IN COWS FED DIETS AT TWO CONCENTRATE TO FORAGE RATIOS WITH OR WITHOUT SBM

Item	Percent concentrate in diet				SE	P
	65		35			
	1	2	1	2		
Organic matter intake, g/d	15372	15162	15316	16984		
Leaving abomasum, g/d						
Total	8205	6670	7461	8913	454.4	.37
Non-microbial	5956	4892	5615	6498	320.6	.38
Ruminal digestion, %						
Unadjusted	46.0	53.7	49.1	42.6	2.6	.24
Adjusted	60.7	65.8	61.8	58.1	1.8	.53
Feces, g/d	6362.6	6812.6	7788.1	8382.7	443.6	.04
Post-ruminal digestion, % of input	60.3^{A}	53.7^{AB}	42.7^{BC}	48.9^{C}	1.4	.01
Total tract digestion	59.5	55.6	48.6	52.1	2.5	.34

^{ABC}Means in a row with different superscripts differ ($P < .05$).

[a]Adjusted for microbial contribution.

Table 8. NITROGEN (N) DIGESTION IN COWS FED DIETS AT TWO CONCENTRATE TO FORAGE RATIOS WITH OR WITHOUT SBM

Item	Percent concentrate in diet				SE	P
	65		35			
	1	2	1	2		
Intake, g/d						
Total	374.7	321.5	400.7	365.6		
SBM	142.9	0	123.3	0		
Leaving abomasum, g/d						
Total	393.3	272.6	336.1	374.5	23.5	.32
Microbial N	206.9	154.4	163.1	204.1	12.5	.35
Ammonia N	22.8	20.3	21.3	41.1	3.4	.14
Non-ammonia, non-microbial	163.6	98.0	151.6	129.4	4.9	.21
Ruminal digestion, %						
Unadjusted	- 1.0	- 17.2	- 15.6	- 1.0	5.5	.54
Adjusted[a]	59.2	69.7	62.4	64.6	2.7	.57
SBM escape, %	45.9		18.0			
Microbial efficiency, gN/kgOM	24.1	17.4	18.7	23.2	1.8	.51
Feces, g/d	192.4	184.6	232.4	233.1	12.9	.06
Post-ruminal digestion, % of input	74.8	69.4	62.2	66.6	1.2	.08
Total tract day, %	52.5	46.5	43.8	43.7	3.2	.66
Expected total tract N digestion, %[b]	69.0	67.4	70.6	68.1		

[a]Adjusted for microbial and ammonia N.

[b]Calc % dig. protein = (.9(%CP)-3)/%CP.

Table 9. COMPOSITION OF BACTERIA HARVESTED FROM RUMEN FLUID IN SBM NITROGEN RUMINAL ESCAPE IN EXPERIMENT

Item	Percent concentrate in diet			
	65		35	
	1	2	1	2
Bacterial nitrogen, % of dry matter	7.7	7.26	7.35	7.08
Bacterial RNA-Nitrogen to total nitrogen	12.8	12.7	12.0	11.6

BIBLIOGRAPHY

Abou-Akkada, A. R., and T. H. Blackburn. 1963. Some observations on the nitrogen metabolsim of rumen proteolytic bacteria. J. Gen. Microbiol. 31:461.

Ahrar, M., and D. J. Schingoethe. 1979. Heat-treated soybean meal as a protein supplement for lactating cows. J. Dairy Sci. 62:932.

Allison, M. J. 1970. Nitrogen metabolism of ruminal microorganisms. pp. 456-473. In: Physiology of Digestion in the Ruminant. A. T. Phillipson (Ed.). Oriel Press, Ltd., New Castle Upon Tyne, England.

Amos, H. E. 1980. Treatment of protein to improve utilization by ruminants. Georgia Nutr. Conf., p. 165.

Annison, E. F., D. Lewis and D. B. Lindsay. 1959. The metabolic changes which occur in sheep transferred to lush spring grasses. I. Changes in blood and rumen constituents. J. Agr. Sci. 53:34.

Annison, E. F., M. I. Chalmers, S. B. M. Marshall, and R. L. M. Synge. 1954. Ruminal ammonia formation in relation to the protein requirement of sheep. III. Ruminal ammonia formation with various diets. J. Agric. Sci. 44:270.

A.O.A.C. 1970. Official methods of analysis (9th Ed.). Association of Official Agricultural Chemists, Washington, D.C.

A.O.A.C. 1975. Official methods of analysis (12th Ed.). Association of Official Agricultural Chemists, Washington, D.C.

A.O.A.C. 1969. Protein dispersion index for determination of bound and unbound protein fractions of vegetable protein sources. Official Method Ba 10-65.

Atlass of nutritional data on United States and Canadian feeds. 1971. National Academy of Sciences, Washington, D.C.

Balch, C. C. and R. C. Campling. 1965. Rate of passage of digesta through the ruminant digestive tract. In Physiological of Digestion in the Ruminant. R. W. Dougherty (Ed.). Butterworth Inc., London.

Bailey, C. B., and C. C. Balch. 1961. Saliva secretion and its relation to feeding cattle. 2. The composition and rate of secretion of mixed saliva in the cow during rest. Br. J. Nutr. 15:383.

Barrio, J. R. 1984. Influence of diet on ruminal in situ disappearance of dry matter and nitrogen. M.S. thesis. Oklahoma State University, Stillwater, OK 74078.

Bartley, E. E. 1976. Bovine saliva: Production and function. pp. 61-71. In: Buffers in Ruminant Physiology and Metabolism. M. S. Weinberg and A. L. Sheffner (Eds.). Church and Dwight Company, Inc., New York, New York.

Bauchop, T., R. T. J. Clarke, and J. C. Newhook. 1975. Scanning electron microscope study of bacteria associated with the rumen epithelium of sheep. Appl. Microbiol. 30,668.

Beever, D. E., D. J. Thomson, and S. B. Cammel. 1976. The digestion of frozen and dried grass by sheep. J. Agric. Sci. 86:443.

Bergen, W. G., D. B. Bates, D. E. Johnson, J. C. Waller and J. R. Black. 1982. Ruminal microbial protein synthesis and efficiency. pp. 99. In: Protein Requirements for Cattle: Symposium. F. N. Owens (Ed.). Okla. State Univ. MP-109.

Bines, J. A. 1976. Regulation of food intake in dairy cows in relation to milk production. Livest. Prod. Sci. 3:115.

Bishop, R. B. 1971. Effect of methionine hydroxy analog on complete lactation of dairy cows. J. Dairy Sci. 54:1240.

Bjarnason, J., and K. J. Carpenter. 1970. Mechanisms of heat damage in proteins. 2. Chemical changes in pure proteins. Br. J. Nutr. 24:313.

Blackburn, T. H. 1968. Protease production by Bacteroides amylophilus strain H 18. J. Gen. Microbiol. 53:27.

Blackburn, T. H. and P. N. Hobson. 1960. Proteolysis in the sheep rumen by whole and fractionated rumen contents. J. Gen. Microbiol. 22:272.

Block, E., L. D. Miller, L. C. Griel, Jr., and D. L. Garwood. 1980. Brown midrib 3 vs. normal corn silage heated vs. unheated soybeans for high producing dairy cows. J. Dairy Sci. 63(Suppl. 1):135. (Abstr.).

Block, C., L. D. Muller, L. C. Griel, Jr., and D. L. Garwood. 1981. Brown midrib-3 corn silage and heat extruded soybeans for early lactating dairy cows. J. Dairy Sci. 64:1813.

Botts, R. L., R. W. Hemken, and L. S. Bull. 1979. Protein reserves in the lactating dairy cows. J. Dairy Sci. 62:433.

Brady, C. J. 1960. Redistribution of nitrogen in grass and leguminous fodder plants during wilting and ensilage. J. Sci. Food Agric. 11:276.

Braund, D. G., K. L. Dolge, R. L. Goings, and R. L. Steele. 1978. Method of formulating dairy cattle rations. U.S. Patent 4, 118, 513.

Broderick, G. A. 1975. Factors affecting ruminant responses to protected amino acids and proteins. pp. 211-259. In: Protein Nutritional Quality of Foods and Feeds. Vol. 1. M. Friedman (Ed.). Marcel Dekker, Inc., New York, New York.

Broderick, G. A. 1978. In vitro procedures for estimating rates of ruminal protein degradation and proportions of protein escaping the rumen undegraded. J. Nutr. 108:181.

Broderick, G. A., and G. T. Lane. 1978. Lactational, in vitro, and chemical evaluation of untreated and formaldehyde-treated casein supplements. J. Dairy Sci. 61:932.

Broderick, G. A. and M. Craig. 1980. Effect of heat treatment on ruminal degradation and escape, and intestinal digestibility of cottonseed meal protein. J. Nutr. 110:2381.

Brookes, I. M. 1984. Effects of formaldehyde-treated and untreated casein supplements on performance of dairy cows offered ryegrass-clover pasture. New Zealand J. of Agricultural Research. 27:491.

Broster, W. H. 1972. Protein requirements of cows for lactation. Pages 293-322 in Handbuch der tierernahrung. Vol. 2 W. Lenkeit and K. Breirem, ed. Paul Parey, Hamburg, Germany.

Broster, W. H., and M. F. Strickland. 1977. Feeding the dairy cow in early lactation. ADAS Quar. Rev. 26:87.

Bryant, M. P. 1977. Microbiology of the rumen. pp. 287-304. In Dukes' Physiology of Domestic Animals. M. J. Swenson (Ed.). Ninth edition, Cornell University Press, Ithaca, New York.

Bryant, M. P., and I. M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bact. 84:605-614.

Bull, Leonard S. 1981. Controlling protein digestion in ruminants. Feedstuffs. 35(31):23-24.

Bull, L. S., M. I. Poos and R. C. Bull. 1977. Protein solubility and NPN for dairy cows--a problem of energy metabolism. Proc. Distillers Feed Res. Conf., p. 23.

Buttery, P. J., and D. J. A. Cole. 1977. Chemical analysis: Sources of error. Proc. Nutr. Soc. 36:211.

Castle, M. E., M. S. Gill and J. N. Watson. 1983. Silage and milk production: a comparison between three rates of high-protein concentrate supplementation of grass silages of two digestibilities. Grass and Forage Sci. 38:135.

Chalmers, M. I., O. P. Cuthbertson, and M. L. M. Synge. 1954. Ruminal ammonia formation in relation to the protein requirement of sheep. I. Duodenal administration and heat processing factors influencing fate of casein supplements. J. Agric. Sci. 44:254.

Chalupa, W. 1972. Metabolic aspects of nonprotein nitrogen utilization in ruminant animals. Fed. Proc. 31:1152.

Chalupa, W. 1975. Rumen bypass and protection of proteins and amino acids. J. Dairy Sci. 58:1198.

Chalupa, W. 1978. Digestion and absorption of nitogenous compounds in ruminants. Proc. 3rd World Congress on Animal Feeding, Madrid, pp. 211-230.

Chaney, A. L. and E. P. Marback. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem., 8:130.

Cheng, K. J. and J. W. Costerton. 1980. In "Digestive physiology and metabolism in ruminants." Ruckelbusch and P. Thevend, eds., MTP Press Ltd., Lancaster, England.

Cheng, K. J. and R. J. Wallace. 1979. The mechanism of passage of endogenous urea through the rumen wall and the role of ureolytic epithelial bacteria in the urea flux. Br. J. Nutr. 42,553.

Clark, J. H. 1975a. Lactation responses to postruminal administration of proteins and amino acids. J. Dairy Sci. 58:1178.

Clark, J. H. 1975b. Nitrogen metabolism in ruminants: Protein solubility and rumen bypass of proteins and amino acids. pp. 261-304. In: Protein Nutritional Quality of Foods and Feeds. Vol. 1. M. Friedman (Ed.). Marcel Dekker, Inc., New York, New York.

Clark, J. H., and B. A. Crooker. 1979. The effects of protein solubility on ruminal protein degradation. pp. 1-26. Proceedings and Events of Brookside Farms Laboratory Associaton, Inc., Annual Jamboree and Conference.

Clark, J. H., and C. L. Davis. 1980. Some aspects of feeding high producing cows. J. Dairy Sci. 63:873.

Clark, J. H., C. L. Davis, and E. E. Hatfield. 1974. Effects of formaldehyde treated soybean meal on nutrient use, milk jield and composition, and free amino acids in the lactating bovine. J. Dairy Sci. 57:1031.

Coleman, G. S. 1967. The metabolism of the amino acids of Escherichia coli and other bacteria by the rumen ciliate Entodinium caudatum. J. Gen. Microbiol. 47: 449.

Cowan, R. T., J. J. Robinson, I. McHattie and K. Pennie. 1981. Effects of protein concentration in the diet on milk yield change in body composition and the efficiency of utilization of body tissue for milk production in ewes. Anim. Prod. 33:111.

Craig, W. M., and G. A. Broderick. 1978. Relationship of N-solubility to ruminal degradation of cottonseed meal protein. In: Abstracts 70th Annual Meeting, American Society of Animal Science. p. 412 (Abstr.).

Crawford, R. J., Jr., W. h. Hoover, C. J. Sniffen, and B. A. Crooker. 1978. Degradation of feedstuff nitrogen in the rumen vs. nitrogen solubility in three solvents. J. Anim. Sci. 46:1768-1775.

Crickenberger, R. G., W. G. Bergen, D. G. Fox and L. A. Gideon. 1979. Effect of protein level in corn-cornsilage diets on abomasal nitrogen passage and utilization by steers. J. Anim. Sci. 49:177.

Crooker, B. A., C. J. Sniffen, W. H. Hoover, and L. L. Johnson. 1978. Solvents for soluble nitrogen measurements in feedstuffs. J. Dairy Sci. 61:437.

Crooker, B. A., J. H. Clark and R. D. Shanks. 1983. Effects of formaldehyde treated dairy soybean meal on milk yield, milk composition, and nutrient digestibility in the diary cow. J. Dairy Sci. 66:492.

Czerkawski, J. W. 1974. Methods for determining 2-6-diaminopimelic acid and 2-amino-ethylphosphonic acid in gut contents. J. Sci. Food Agr. 25:45.

Czerkawski, J. W. 1978. Reassessment of efficiency of synthesis of microbial matter in the rumen. J. Dairy Sci. 61:1261.

Danke, R. J., L. B. Sherrod, E. C. Nelson and A. D. Tillman. 1966. Effects of autoclaving and steaming of cottonseed meal for different lengths of time on nitrogen solubility and retention in sheep. J. Anim. Sci. 25:181.

Davis, R. F. 1978. Response of dairy cattle to ration protein of different solubilities. Proc. Maryland Nutrition Conf., pp. 116-120.

Demeijer, D. and C. J. Van Nevel. 1976. A critical approach to isotope methods for measuring microbial growth in the rumen in vitro. In: Tracer Studies on Nonprotein Nitrogen for Ruminants III, IAEA, Vienna. p. 637.

Driedger, A. and E. E. Hatfield. 1972. Influence of tannins on the nutritive value of soybean meal for ruminants. J. Anim. Sci. 34:465.

Duncan, C. W., J. P. Agrawala, C. F. Huffman and R. W. Luecke. 1953. A quantitive study of rumen synthesis in the bovine on natural and purified rations. II. Amino acid content of mixed rumen proteins. J. Nutr. 49:41.

Egan, A. R. 1965. Nutritional status and intake regulation in sheep. III. The relationship between improvement of nitrogen status and increase in voluntary intake of low-protein roughages by sheep. Aust. J. Agr. Res. 16:43.

Ellis, W. C., C. Lascano, R. Teeter and F. N. Owens. 1982. Solute and particulate flow markers, p. 37. In: Protein Requirements for Cattle: Symposium. F. N. Owens (Ed.). Okla. State Univ., MP-109.

Erdman, R. A., E. Russek, G. Switalski and J. H. Vandersall. 1982. Prediction of rumen protein degradation using estimates of rates of degradation in lactating dairy cows. In: Abstracts 74th Annual Meeting, American Society of Animal Science. 55:419 (Abstr.).

Erdman, R. A. and J. H. Vandersall. 1983. Effect of rumen protein degradability on milk yield of dairy cows in early lactation. J. Dairy Sci. 66:1873.

Faichney, G. J. 1971. The effect of formaldehyde-treated casein on the growth of ruminant lambs. Aust. J. Agric. Res. 22:453.

Fenton, T. W. and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and feces. Can. J. Anim. Sci. 59:631.

Ferguson, K. A. 1975. Protection of dietary proteins in the rumen. pp. 448-464. In: Digestion and Metabolism in the Ruminant. I. W. McDonald and A. C. I. Warner (Eds.). University of New England Publishing Unit, Armidale, Australia.

Finley, J. W. and M. Friedman. 1973. Chemical methods for available lysine. Cereal Chem. 50:101.

Flores, J. F., T. H. Stobbs, and D. J. Minson. 1979. Influence of the legume leucaena leucocephala and formal-casein on the production and composition of milk from grazing cows. J. Agric. Sci. Camb. 92:351.

Foldager, J., and J. T. Huber. 1979. Influence of protein percent and source in cows in early lactation. J. Dairy Sci. 52:954.

Fontaine, T. D. and R. S. Burnette. 1944. Peptization of nitrogenous constituents of solvent-extracted peanut meal. Ind. Eng. Chem. 36:164.

Fraenkel-Conrat, H., and H. S. Olcott. 1948. The reaction of formaldehyde with proteins. V. Cross-linking between amino acid primary amide or quanidyl groups. J. Anim. Chem. Soc. 70:2673.

Garrett, J. E., R. D. Goodrich and J. C. Meiske. 1982. Measurement of bacteria nitrogen using D-alanine. p. 23. In: Protein Requirements for Cattle: Symposium. F. N. Owens (Ed.). Okla. State Univ., MP-109.

Glimp, H. H., M. R. Karr, C. O. Little, P. W. Woolfolk. G. E. Mitchell, Jr., and L. W. Hudson. 1967. Effect of reducing soybean protein solubility by dry heat on the protein utilization of young lambs. J. Anim. Sci. 26:858.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses. Agriculture Handbook No. 379.

Goering, H. K., and D. R. Waldo. 1974. Processing effects on protein utilization by ruminants. pp. 25-35. Proc. Cornell Nutrition Conf.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agric. Res. Serv., U.S. Dep. Agric. Handbook No. 379.

Goetsch, A. L., and F. N. Owens. 1985a. Effects of sarsaponin on digestion and passage rates in cattle medium to low concentrates. J. Dairy Sci. 68:2377.

Goetsch, A. L., and F. N. Owens. 1985b. The effects of commercial processing method of cottonseed meal on site and extent of digestion in cattle. J. Anim. Sci. 60:803.

Griel, L. C., Jr., R. A. Patton, R. D. McCarthy, and P. Chandler. 1968. Milk production response to feeding methionine hydroxy analog to lactating dairy cows. J. Dairy Sci. 51:1866.

Grieve, D. G., E. Wheeler, Y. Yu and G. K. Macleod. 1980. Effects of dry or ensiled feeds and protein percent on milk production and nitrogen utilization by lactating cows. J. Dairy Sci. 63:1282.

Grummer, R. R. and J. H. Clark. 1982. Effect of dietary nitrogen solubility on lactation performance and protein and dry matter degradation in situ. J. Dairy Sci. 65:1432.

Ha, J. K. and J. J. Kennelly. 1984. In situ dry matter and protein degradation of various protein sources in dairy cattle. Can. J. Anim. Sci. 64:443.

Harrison, D. G. and A. B. McAllan. 1980. Factors affecting microbial growth yields in the reticulo-rumen. p. 205. In: Digestive Physiology and Metabolism in Ruminants. Y. Ruckebusch and P. Thivend (Eds.). AVI Publishing Co., Westport, Connecticut.

Hatfield, E. E. 1973. Treating dietary proteins with tannins or aldehydes. In: Effect of Processing on the Nutritive Value of Feeds, National Academy of Sciences, Washington, D.C.

Hendrickx, H. K., D. I. Demeijer and C. J. Van Nevel. 1972. The pool of free amino acids in rumen liquor. In: 2nd World Congress of Animal Feeding. 2. Free Communications, Madrid. p. 51.

Hendrickx, H., and J. Martin. 1963. In vitro studies of the nitrogen metabolism in the rumen. Compt. Rend. Res. Sci. Ind. Agr., Bruxelles. 31:1-43.

Hespell, R. B. and M. P. Bryant. 1979. Efficiency of rumen microbial growth: Influence of some theoretical and experimental factors on Y_{ATP}. J. Anim. Sci. 49:1640.

Higgenbotham, G. E., N. P. Johnston and D. Andrus. 1984. Effect of nonprotein nitrogen supplementation of highly insoluble dairy rations on the performance of high producing dairy cows. J. Dairy Sci., Suppl. 1, 67:120.

Hogan, J. P. 1975. Quantitative aspects of nitrogen utilization in ruminants. J. Dairy Sci. 58:1164.

Hogan, J. P. and J. H. Hemsley. 1976. Protein digestion in the rumen. pp. 73-77. In: From Plant to Animal Protein. T. M. Sutherland, J. R. McWilliams, and R. A. Leng (Eds.). University of New England Publishing Unit, Armidale, Australia.

Houpt, T. R. 1970. Transfer of urea and ammonia to the rumen. pp. 119-131. In: Physiology of Digestion and Metabolism in the Ruminant. A. T. Phillipson (Ed.). Oriel Press, Ltd., Newcastle upon Tyne, England.

Huber, J. T. and L. Kung, Jr. 1981. Protein and nonprotein nitrogen utilization in dairy cattle. J. Dairy Sci. 64:1170.

Hudson, L. W., H. A. Glimp, C. O. Little and P. G. Woolfolk. 1970. Ruminal and post-ruminal nitrogen utilization by lambs fed heated soybean meal. J. Anim. Sci. 30:609.

Hume, J. D. 1974. The proportion of dietary protein escaping degradation in the rumen of sheep fed on various protein concentrates. Aust. J. Agr. Res. 25:155.

Hungate, R. E. 1966. The Rumen and its Microbes. Academic Press, New York, York.

Hvelplund, T., P. D. Moller, J. Nadsen and M. Hesselholt. 1976. Flow of digesta through the gastro-intestinal tract in the bovine with special reference to nitrogen. Royal Vet. and Agr. Univ. (Denmark), Yearbook 1976:173.

Isaacs, J. and F. N. Owens. 1972. Protein soluble in rumen fluid. J. Anim. Sci. 35:267 (Abstr.).

John, A., H. R. Isaacson, and M. P. Bryant. 1974. Isolation and characteristics of a ureolytic strain of Selenomonas ruminantium. J. Dairy Sci. 57:1003.

Jones, G. A. 1967. Ureolytic rumen bacteria. pp. 111-124. Urea as a Protein Supplement. M. H. Briggs (Ed.). Pergamon Press, Ltd., New York, New York.

Jones, G. A., R. A. MacLeod, and A. C. Blackwood. 1964. Ureolytic rumen bacteria. I. Characteristics of the microflora from a urea fed sheep. Can. J. Microbiol. 10:371.

Kennedy, P. M., J. R. Lindsay and L. P. Milligan. 1980. In vivo measurement of rumen microbial growth using ^{35}S. J. Agric. Sci. (Camb.). 94:89.

Krishnamoorthy, U., T. V. Muscato, C. J. Sniffen and P. J. Van Soest. 1982. Nitrogen Fractions in Selected Feedstuffs. J. Dairy Sci. 65:217.

Kung, L., Jr., and J. T. Huber. 1983. Performance of high producing cows in early lactation fed protein of varying amounts, sources, and degradability. J. Dairy Sci. 66:227.

Kung, L., Jr., J. T. Huber and L. D. Satter. 1983. Influence of nonprotein nitrogen and protein of low rumen degradability on nitrogen flow and utilization in lactating dairy cows. J. Dairy Sci. 66:1863.

Kung, L., Jr., J. T. Huber, W. G. Bergen and D. Petitclerc. 1984. Amino acids in plasma and duodenal digesta and plasma growth hormone in cows fed varying amounts of protein of differing degradability. J. Dairy Sci. 67:2519.

Laughren, L. C. 1978. Effects of feeding diets containing increasing levels of crude protein on the quantity and quality of nitrogenous compounds reaching the duodenum of sheep. M.S. thesis, University of Wisconsin, Madison, Wisconsin.

Lehninger, A. L. 1975. Biochemistry (2nd. ed.). Worth Publishers, Inc., New York.

Leibholtz, J. 1965. The free amino acids occurring in the blood plasma and rumen liquor of the sheep. Aust. J. Agr. Res. 16:973.

Lewis, D. 1957. Blood-urea concentration in relation to protein utilization in the ruminant. J. Agric. Sci. 44:270.

Lewis, T. R. 1962. The interrelationships of individual proteins and carbohydrates during fermentation of starch in the presence of proteins and other substances containing nitrogen. J. Agr. Sci. 58:73.

Lewis, T. R. and R. S. Emery. 1962. Intermediate products in the catabolism of amino acids by rumen organisms. J. Dairy Sci. 45:1363.

Lindberg, J. E., H. S. Solinan, and S. Sanne. 1982. A study of the rumen degradability of untreated and heat-treated rape seed meal and of whole rape seed, including a comparison between two nylon bag techniques. Swed. J. Agric. Res. 12:83.

Little, C. O., W. Burroughs, and W. Woods. 1963. Nutritional significance of soluble nitrogen in dietary proteins for ruminants. J. Anim. Sci. 22:358.

Little, C. O., and G. E. Mitchell, Jr. 1967. Abomasal vs. oral administration of proteins to wethers. J. Anim. Sci. 26:411.

Loerch, S. C. 1982. Factors affecting degradation and utilization of protein sources by ruminants. Ph.D. Dissertation. Univ. of Illinois, Urbana, Illinois.

Loerch, S. C., L. L. Berger, D. Glanola and G. C. Fahey. 1983a. Effects of dietary protein source and energy level on in situ nitrogen disappearance of various protein sources. J. Anim. Sci. 56:206.

Loerch, S. C., L. L. Berger, D. Plegge and G. C. Fahey, Jr. 1983b. Digestibility and rumen bypass of soybean meal, blood meal, meat and bone meal and dehydrated alfalfa N. J. Anim. Sci. 55(Suppl. 1):437.

Loerch, S. C., L. L. Berger, D. Plegge and G. C. Fahey. 1983c. Digestibility and rumen escape of soybean meal, meat and bone meal and dehydrated alfalfa nitrogen. J. Anim. Sci. 57:1037.

Loosli, J. K., J. M. Elliot and R. G. Warner. 1960. Comparative value of corn distillers dried grains with solubles, soybean oil meal and linseed oil meal for milk production. J. Dairy Sci. 43:816.

Loosli, J. K., and R. G. Warner. 1958. Distillers grains, brewers grains and urea as protein supplements for dairy rations. J. Dairy Sci. 41:1446.

Loosli, J. K., R. G. Warner, and H. F. Hintz. 1961. Value of corn distillers dried grains, soybean oil meal, heated soybeans and soybean oil meal plus starch for milk production. J. Dairy Sci. 44:1919.

Loosli, J. K., H. H. Williams, W. E. Thomas, F. H. Ferris and L. A. Maynard. 1949. Synthesis of amino acids in the rumen. Science. 110:144.

Lucas, H. L. 1956. Switchback trials for more than two treatments. J. Dairy Sci. 39:146.

MacRae, J. C. 1976. Utilization of the protein of green forage by ruminants at pasture. pp. 93-97. In: From Plant to Animal Protein. T. M. Sutherland, J. R. McWilliam, and R. A. Leng (Eds.). University of New England Publishing Unit, Armidale, Australia.

Mahadevan, S., I. D. Erfle and F. D. Sauer. 1980. Degradation of soluble and insoluble proteins by Bacteroides amylophilus protease and by rumen microorganisms. J. Anim. Sci. 50:723.

Majdoub, A., G. T. Lane, and T. E. Aitchison. 1978. Milk production response to nitrogen solubility in dairy rations. J. Dairy Sci. 61:59.

Mangan, J. L. 1972. Quantitative studies on nitrogen metabolism in the bovine rumen. The rate of proteolysis of casein and ovalbumin and the release and metabolism of free amino acids. Br. J. Nutr. 27:261.

Mathers, J. C., C. M. Horton, and E. L. Miller. 1977. Rate and extent of protein degradation in the rumen. Proc. Nutr. Soc. 36:37a. (Abstr.).

McAllan, A. B. and R. H. Smith. 1969. Nucleic acid metabolism in the ruminant. Determination of nucleic acids in digesta. Brit. J. Nutr. 23:671.

McGuffy, R. K., D. J. Schingoethe and J. L. Hattan. 1980. Sunflower seeds for lactating dairy cows. J. Dairy Sci. 63(Suppl 1):155 (Abstr.).

McLeod, M. N. 1974. Plant tannins--their role in forage quality. Nutrition Abstracts and Reviews. 44:803.

Mehrez, A. Z., and E. R. Ørskov. 1977. A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. Agric. Sci. 88:645.

Merchen, N., T. Hanson and T. Klopfenstein. 1979. Ruminal bypass of brewers dried grains protein. J. Anim. Sci. 49:192.

Mertens, D. R. 1977. Importance and measurement of protein insolubility in ruminant diets. Proc. Georgia Nutrition Conf. pp. 30-41.

Mielke, C. D., and D. J. Schingoethe. 1979. Heat treated soybean meal for cows in early lactation. J. Dairy Sci. (Suppl. 1):74(Abstr.).

Mielke, C. D. and D. J. Schingoethe. 1981. Heat-treated soybeans for lactating cows. J. Dairy Sci. 64:1579.

Miller, E. L. 1982. Methods of assessing proteins for ruminants, including laboratory methods. In: E. L. Miller and I. H. Pike (Ed.). Protein contribution of feedstuffs for ruminants: application to feed formulation, p. 18. Butterworth Scientific, London.

Mohamed, O. E., and R. H. Smith. 1977. Measurement of protein degradation in the rumen. Proc. Nutr. Soc. 36:152A. (Abstr.).

Mueller, L. D., D. Rodriquez, and D. J. Schingoethe. 1975. Formaldehyde treated whey protein concentrate for lactating dairy cattle. J. Dairy Sci. 58:1847.

Mustakas, G. C. 1980. Recovery of oil from soybeans. In: D. R. Erickson (Ed.), Handbook of Soy Oil Processing and Utilization, pp. 49-65. American Soybean Assoc., St. Louis, MO, and American Oil Chemists Soc., Champaign, IL.

National Research Council. 1976. Nutrient requirements of domestic animals, No. 4 nutrient requirements of beef cattle. Fifth revised ed. National Academy of Sciences National Research Council. Washington, D.C.

National Research Council. 1978. Nutrient requirements of dairy cattle. Fifth revised ed. National Academy of Sciences National Research Council, Washington, D.C.

National Research Council. 1985. Ruminant nitrogen usage. National Academy of Sciences National Research Council, Washington, D.C.

Netemeyer, D. T., L. J. Bush, J. W. Ward and S. A. Jafri. 1982. Effect of heating soybean meal for dairy cows. J. Dairy Sci. 65:235.

Neudoerffer, T. S., P. A. Leadbeater, F. D. Horney and H. S. Bayley. 1971. The influence of level of grain intake on protein digestion in the intestine of cattle. Brit. J. Nutr. 25:343.

Nishimuta, J. F., D. G. Ely and J. A. Boling. 1972. Nitrogen metabolism in lambs fed soybean meal treated with heat, formalin and tannic acid. J. Nutr. 103:49.

Nocek, J. E., K. A. Cummins, and C. E. Polan. 1979. Ruminal disappearance of crude protein and dry matter in feeds and combined effects in formulated rations. J. Dairy Sci. 62:1587.

Nugent, J. H. A. and J. L. Mangan. 1978. Rumen proteolysis of fraction I leaf protein, casein and bovine serum albumin. Proc. Nutr. Soc. 37:29A.

Okeke, G. C., J. G. Buchanan-Smith and W. I. Grovum. 1983. Effects of buffers on ruminal rate of passage and degradation of soybean meal in steers. J. Animal Sci. 56:1393.

Oldham, J. D. 1977. Protein degradation in ruminants in relation to protein need. pp. 9-13. Process Biochemistry, September, 1977.

Oltjen, R. R. 1969. Effects of feeding ruminants non-protein nitrogen as the only nitrogen source. J. Anim. Sci. 28:673.

Ørskov, E. R. 1985. Personal communication. Intragastric nutrition of ruminants. Rowett Research Institute, Aberdeen, Scotland. Amer. Soc. of An. Sci. 68:829.

Ørskov, E. R., and A. Z. Mehrez. 1977. Estimates of extent of protein degradation from basal feeds in the rumen of sheep. Proc. Nutr. Soc. 36:78A.

Ørskov, E. R., and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499.

Ørskov, E. R., G. W. Reid and I. McDonald. 1981. The effects of protein degradability and food intake on milk yield and composition in cows in early lactation. Br. J. Nutr. 45:547.

Owens, F. N. 1978. Protein solubility and ruminant nutrition. Feedstuffs. 50(28):23-24,39.

Pankhurst, I. M., I. B. Robinson and G. L. Mathews. 1980. The effects of feeding dairy cows formaldehyde protected decorticated sunflower seed supplement with and without added soybean meal. Australian J. of Dairy Technology. 35:9.

Paulsen, T. M. 1968. Protein dispersibility index (PDI). J.A.O.C.S. 37:165.

Pearson, I. M., and J. A. B. Smith. 1943. The utilization of urea in the bovine rumen. 3. The synthesis and breakdown of protein in rumen digesta. Biochem. J. 37:153.

Peter, A. P., E. E. Hatfield, F. N. Owens, and U. S. Garrigus. 1971. Effects of aldehyde treatments of soybean meal on in vitro ammonia release, solubility, and lamb performance. J. Nutr. 101:605.

Phillipson, A. T., and J. L. Mangan. 1959. Bloat in cattle. XVI. Bovine saliva: The chemical composition of the parotid, submaxillary, and residual secretions. N. Z. J. Agric. Res. 2:990.

Pichard, G., and P. J. Van Soest. 1977. Protein solubility of ruminant feeds. pp. 91-98. Proc. Cornell Nutrition Conference.

Pilgrim, A. F., F. V. Gray, R. A. Weller and C. B. Belling. 1970. Synthesis of microbial protein from ammonia in the sheep's rumen and the proportion of dietary nitrogen converted into microbial nitrogen. Brit. J. Nutr. 24:589.

Plegge, S. D., L. L. Berger and G. C. Fahey, Jr. 1982. The effect of roasting on the utilization of soybean meal by ruminants. J. Anim. Sci. 55:126.

Poos-Floyd, M., T. Klopfenstein, and R. A. Britton. 1984. Evaluation of laboratory techniques for predicting ruminal protein degradation. J. Dairy Sci. 68:829.

Reid, J. T., and J. Robb. 1971. Relationships of body composition to energy intake and energetic efficiency. J. Dairy Sci. 54:553.

Reis, P. J., and D. A. Tunks. 1969. Evaluation of formaldehyde-treated casein for wool growth and nitrogen retention. Aust. J. Agric. Res. 20:775.

Rodgers, J. A., B. C. Marks, C. L. Davis and J. H. Clark. 1979. Alteration of rumen fermentation in steers by increasing rumen fluid dilution rate with mineral salts. J. Dairy Sci. 62:1599.

Russell, J. B., and S. A. Martin. 1984. Effects of various methane inhibitors on the fermentation of amino acids by mixed rumen microorganisms in vitro. J. Anim. Sci. 59:1329-1338.

Russell, J. B., W. M. Sharp and R. L. Baldwin. 1979. The effect of pH on maximum bacterial growth rate and its possible role as a determinant of bacterial competition in the rumen. J. Anim. Sci. 48:251.

Sachteleben, S. S. 1980. Studies on the effect of diet on nitrogen passage to the lower gastrointestinal tract in steers. Ph.D. thesis, Michigan State University, East Lansing.

Sahlu, T., D. J. Schingoethe and A. K. Clark. 1984. Lactational and chemical evaluation of soybean meals heat-treated by two methods. J. Dairy Sci. 67:1725.

Santos, K. A., M. D. Stern and L. D. Satter. 1984. Protein degradation in the rumen and amino acid absorption in the small intestine of lactating dairy cattle fed various protein sources. J. Anim. Sci. 58:244.

Satter, L. D., G. P. Brooke, and C. G. Schwab. 1970. Effect of formaldehyde-treated soybean oil meal on performance of growing lambs and lactating cows. J. Dairy Sci. 53:688. (Abstr.).

Satter, L. D., and R. E. Roffler. 1975. Nitrogen requirement and utilization in dairy cattle. J. Dairy Sci. 58:1219.

Satter, l. D., L. W. Whitlow, and G. L. Beardsley. 1977. Resistance of protein to rumen degradation and its significance to the dairy cow. pp. 63-72. Distillers Feed Research Council Proceedings, Cincinnati, Ohio.

Schelling, G. T. and E. E. Hatfield. 1967. Abomasal infusion of casein to lambs fed a purified diet. J. Anim. Sci. 26:929.

Schelling, G. T., S. E. Koenig and T. C. Jackson. 1982. Nucleic acids and purine or pyrimidine bases as markers for protein synthesis in the rumen. p. 1. In: Protein Requirements for Cattle: Symposium. F. N. Owens (Ed.). Okla. State Univ., MP-109.

Schingoethe, D. J. 1984. Application of ruminant bypass proteins in ruminant diets. American Soybean Association Quarterly Publication-APRIL.

Schingoethe, D. J. and M. Ahrar. 1979. Protein solubility, amino acid composition, and biological value of regular and heat-treated soybean and sunflower meals. J. Dairy Sci. 62:925.

Schwab, C. G., L. D. Atter, and A. B. Clay. 1976. Response of lactating dairy cows to abomasal infusion of amino acids. J. Dairy Sci. 59:1254.

Sengar, S. S. and V. D. Mudgal. 1983. Effect of reducing solubility of groundnut cake protein on the water and mineral metabolism of lactating goats. Indian J. Dairy Sci. 36:(1):79.

Sharma, H. R., J. R. Ingalls, and R. J. Parker. 1974. Effects of treating rapeseed meal and casein with formaldehyde on the flow of nutrients through the gastrointestinal tract of fistulated Holstein steers. Can. J. Anim. Sci. 54:305.

Sherrod, L. B., and A. D. Tillman. 1962. Effects of varying processing temperatures upon the nutritive values for sheep of solvent-extracted soybean and cottonseed meals. J. Anim. Sci. 21:901.

Sherrod, L. B. and A. D. Tillman. 1964. Further studies on the effects of different processing temperatures on the utilization of solvent extracted cottonseed protein by sheep. J. Anim. Sci. 23:510.

Smith, R. H. 1975. Nitrogen metabolism in the rumen and the composition and nutritive value of nitrogen compounds entering the duodenum. pp. 399-415. In: Digestion and Metabolism in the Ruminant. I. W. McDonald and A. C. I. Warner (Eds.). University of New England Publishing Unit, Armidale, Australia.

Smith, R. H., A. B. McAllan, D. Hewitt and P. E. Lewis. 1978. Estimation of amounts of microbial and dietary nitrogen compounds entering the duodenum of cattle. J. Agr. Sci. (Camb.). 90:557.

Snedecor, G. W., and W. G. Cochran. 1980. Statistical Methods. The Iowa State University Press, Ames, Iowa.

Sniffen, C. J. 1974. Nitrogen utilization as related to solubility of NPN and protein in feeds. pp. 12-18. Proc. Cornell Nutrition Conf.

Somers, M. 1961a. Factors influencing the secretion of nitrogen in sheep saliva. I. The distribution of nitrogen in the mixed and parotid saliva of sheep. Aust. J. Exp. Biol. 39:111.

Somers, M. 1961b. Factors influencing the secretion of nitrogen in sheep saliva. 2. The influence of nitrogen intake upon blood urea nitrogen and upon the total nitrogen and urea nitrogen in the parotid saliva of sheep. Aust. J. Exp. Biol. 39:123.

Spears, J. W., E. E. Hatfield and J. H. Clark. 1980. Influence of formaldehyde treatment of soybean meal on performance of growing steers and protein availability in the chick. J. Anim. Sci. 50:750.

Spears, J. W., J. H. Clark, and E. E. Hatfield. 1979. Nitrogen utilization by steers fed formaldehyde-treated soybean meal. 71st Annual Meeting, American Society of Animal Science. p 406(Abstr.).

Statistical Analysis System. 1979. SAS User's Guide. SAS Institute Inc., Gary, North Carolina.

Stern, M. D., M. E. Ortega and L. D. Satter. 1983. Retention time in rumen and degradation of protein supplements fed to lactating dairy cattle. J. Dairy Sci. 66:1264.

Stern, M. D. and L. D. Satter. 1982. In vivo estimation of protein degradability in the rumen. In: Protein Requirements of Cattle: Symposium. F. N. Owens, Ed., Okla. State Univ., Misc. Publ. MP-109:57.

Stern, M. D., K. A. Santos, and L. D. Satter. 1985. Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle fed heat-treated whole soybeans. J. Dairy Sci. 68:45-56.

Stern, M. D. and W. H. Hoover. 1979. Methods for determining and factors affecting rumen microbial protein synthesis. A review. J. Anim. Sci. 49:1590.

Stobbs, T. H., D. J. Minson, and M. N. Mcleod. 1977. The response of dairy cows grazing nitrogen fertilized grass pasture to a supplement of protected casein. J. Agric. Sci. Camb. 89:137.

Stock, R., N. Merchen, T. Klopfenstein and M. Poos. 1981. Feeding value of slowly degraded proteins. J. Anim. Sci. 53:1109.

Sutherland, T. M. 1976. The overall metabolism of nitrogen in the rumen. pp. 65-72. In: From Plant to Animal Protein. T. M. Sutherland, I. R. McWilliam, and R. A. Leng (Eds.). University of New England Publishing Unit, Armidale, Australia.

Sutton, J. D. and J. D. Oldham. 1977. Feed evaluation by measurement of sites of digestion in cannulated ruminants. Proc. Nutr. Soc. 36:203.

Tagari, H., I. Ascarelli, and A. Bondi. 1962. The influence of heating on the nutritive value of soya-bean meal for ruminants. Br. J. Nutr. 16:237.

Tamminga, S. 1979. Protein degradation in the forestomachs of ruminants. J. Anim. Sci. 49:1615.

Tamminga, S., C. J. van der Koelen and A. M. van Vuuren. 1979. Effect of the level of feed intake on nitrogen entering the small intestine of dairy cows. Livestock Prod. Sci. 6:255.

Teeter, R. G. 1981. Indigestible markers: methodology and applications in ruminant nutrition. Ph.D. Dissertation. Oklahoma State University, Stillwater, OK 74078.

Theurer, C. B. 1979. Microbial protein synthesis as influenced by diet. p. 97. In: Regulation of Acid-Base Balance. L. C. Hallman and R. W. Rice (Ed.). Church and Dwight Co., Inc., Nutley, New Jersey.

Theurer, C. B. 1982. Microbial protein estimation using DAP, AEP and other amino acids as markers. p. 10. In: Protein Requirements for Cattle: Symposium. F. N. Owens (Ed.). Okla. State Univ., MP-109.

Theurer, C. B. 1982. Microbial protein estimation using DAP, AEP and other amino acids as markers. p. 10. In: Protein Requirements for Cattle: Symposium. F. N. Owens (Ed.). Okla. State Univ., MP-109.

Thomas, E., A. Trenkle and W. Burroughs. 1979a. Evaluation of protective agents applied to soybean meal and fed to cattle. I. Laboratory measurements. J. Anim. Sci. 49:1337.

Thomas, E., A. Trenkle and W. Burroughs. 1979b. Evaluation of protective agents applied to soybean meal and fed to cattle. II. Feedlot trials. J. Anim. Sci. 49:1346.

Thomson, D. J., D. E. Beever, D. C. Mundell, M. L. Elderfield and D. G. Harrison. 1975. The effect of altering dilution rate on the pattern of fermentation in the rumen. Proc. Nutr. Soc. 34:111A.

Tillman, A. D., and K. S. Sidhu. 1969. Nitrogen metabolism in ruminants: Rate of ruminal ammonia production and nitrogen utilization by ruminants. A Review. J. Anim. Sci. 28:689.

Uden, P., R. Parra, and P. J. Van Soest. 1974. Factors influencing the reliability of the nylon bag technique. J. Dairy Sci. 57:622. (Abstr.).

Van Buren, J. P., and W. B. Robinson. 1969. Formation of complexes between protein and tannic acid. J. Agric. Food Chem. 17:772.

Van der Aar, P. J., L. L. Berger and G. C. Fahey, Jr. 1982. The effect of alcohol treatments on solubility and in vitro and in situ digestibilities of soybean meal protein. J. Anim. Sci.

Vandersall, J. H. and R. A. Erdman. 1984. Effect of dietary protein and degradability on milk yield: early lactation. J. Dairy Sci., Suppl. 1, 67:113.

Van Dijk, H. J., G. D. O'Dell, P. R. Perry and L. W. Grimes. 1983. Extruded versus raw ground soybeans for dairy cows in early lactation. J. Dairy Sci. 66:2521.

Van Hellen, R. W., and W. C. Ellis. 1977. Sample container porosites for rumen in situ studies. J. Anim. Sci. 44:141-146.

Verite, R., and M. Journet. 1977. Utilization des tourteaux traites au formol par les vaches laitieres. II. Effects sur la production laitiere du traitement des tourteaux au formol et du niveau d'apport ayote au debut de la lactation. Ann. Zootech. 26:183.

Vicente, G. R., J. A. Shelford, R. G. Peterson, and C. R. Krishnamurti. 1984. Effects of feeding canola-meal-protected tallow or soybean-meal-protected tallow in the low-roughage diet of dairy cows in early lactation. Can. J. Anim. Sci. 64:81.

Virtanen, A. I. 1969. On nitrogen metabolism in milking cows. Fed. Proc. 23:232.

Waldo, D. R., and H. K. Goering. 1979. Insolubility of proteins in ruminant feeds by four methods. J. Anim. Sci. 49:1560.

Waller, J. C. 1978. Slowly degraded protein sources for ruminants. Ph.D. Dissertation. Univ. of Nebraska, Lincoln.

Wallis, O. C., and G. S. Coleman. 1967. Incorporation of ^{14}C-labeled components of Escherichia coli and of amino acids by Isotricha intestinalis and Isotricha prostoma from the sheep rumen. J. Gen. Microbiol. 40:49:315.

Warner, R. G., J. K. Loosli, and R. F. Davis. 1957. A study of the value of corn distillers dried grains, coconut oil meal and corn gluten fed for milk production. J. Dairy Sci. 40:123.

Weakley, D. C. 1981. Personal communication. Checker Board Square, St. Louis, MO.

Weakley, D. C. 1983. Influence of roughage level, ruminal pH and ammonia concentration on ruminal protein degradation and microbial synthesis in cattle. Ph.D. Thesis. Oklahoma State University, Stillwater, OK 74078.

Weller, R. A., F. V. Gray and A. F. Pilgrim. 1958. The conversion of plant nitrogen to microbial nitrogen in the rumen of sheep. Brit. J. Nutr. 12:421.

Wilson, G. F. 1970. The influence of protein supplements on milk yield and composition. Proc. N. Z. Soc. Anim. Prod. 30:123.

Wohlt, J. E., C. J. Sniffen, and W. H. Hoover. 1973. Measurement of protein solubility in common feedstuffs. J. Dairy Sci. 56:1052.

Wohlt, J. E., C. J. Sniffen, W. H. Hoover, L. L. Johnson, and C. K. Walker. 1976. nitrogen metabolism in wethers as affected by dietary protein solubility and amino acid profile. J. Anim. Sci. 42:1280.

Zinn, R. A. and F. N. Owens. 1982. Rapid procedure for quantifying nucleic acid content of digesta. p. 26. In: Protein Requirements for Cattle: Symposium. F. N. Owens (Ed.). Okla. State Univ., MP109.

Zinn, R. A. and F. N. Owens. 1983. Site of protein digestion in steers: Predictability. J. Anim. Sci. 56:707.

Zinn, R. A., L. S. Bull and R. W. Hemken. 1981. Degradation of supplemental proteins in the rumen. J. Anim. Sci. 52:857.

Yang, C-M. J., D. J. Schingoethe, C. R. Mueller, and D. P. Casper. 1984. Protected methionine and heat-treated soybean meal for high-producing diary cows. J. Dairy Sci. 67:(Suppl.), p. 54:132.

Yu, Y. 1978. Effect of treating full-fat whole soybeans with dry heat or formaldehyde on digestibilities of nitrogen and polyenic acids. J. Dairy Sci. 61:128.

APPENDIX A

CORRELATION COEFFICIENTS, DRY MATTER DISAPPEARANCE AND NITROGEN DISAPPEARANCE PLOTS OF FULL-FAT EXTRUDED AND 44% SOLVENT EXTRACTED SOYBEAN MEAL EXAMINED IN CHAPTER III

SAS

CORRELATION COEFFICIENTS / PROB > |R| UNDER HO RHO 0 / N = 56

	TRT	DMO	DM4	DM12	DM20	PRTO	PRT4	PRT12	PRT20	PIN	NSOL	DIE	STEAM
TRT	1 00000 0 0000	0 47665 0 0002	0 29287 0 0285	0 00712 0 9585	-0 40123 0 0022	0 42405 0 0011	0 38050 0 0038	0 35732 0 0069	-0 00158 0 9908	-0 01114 0 9351	0 41914 0 0013	-0 81860 0 0001	0 21483 0 1118
DMO	0 47665 0 0002	1 00000 0 0000	0 80054 0 0001	0 34257 0 0098	-0 15307 0 2600	0 85125 0 0001	0 76210 0 0001	0 51632 0 0001	-0 01530 0 9109	0 17157 0 2061	0 37937 0 0039	-0 50090 0 0001	-0 01990 0 8842
DM4	0 29287 0 0285	0 80054 0 0001	1 00000 0 0000	0 63107 0 0001	0 14383 0 2902	0 74269 0 0001	0 86890 0 0001	0 65475 0 0001	0 06640 0 6268	-0 15462 0 2552	0 38868 0 0031	-0 41805 0 0013	0 01739 0 8988
DM12	0 00712 0 9585	0 34257 0 0098	0 63107 0 0001	1 00000 0 0000	0 44430 0 0006	0 58918 0 0001	0 25768 0 0552	0 63885 0 0001	-0 07119 0 6021	-0 47234 0 0002	0 47915 0 0002	-0 27924 0 0371	-0 33595 0 0111
DM20	-0 10123 0 0072	-0 15307 0 2600	0 14383 0 2902	0 44430 0 0006	1 00000 0 0000	0 12869 0 3445	-0 04045 0 7672	0 02234 0 8702	0 62495 0 0001	-0 41413 0 0015	0 15273 0 2611	0 20685 0 1261	-0 24461 0 0692
PRTO	0 42405 0 0011	0 85125 0 0001	0 74269 0 0001	0 58918 0 0001	0 12869 0 3445	1 00000 0 0000	0 59558 0 0001	0 64361 0 0001	0 04374 0 7489	-0 09670 0 4783	0 67083 0 0001	-0 52153 0 0001	-0 04392 0 7479
PRT4	0 38050 0 0038	0 76210 0 0001	0 86890 0 0001	0 25768 0 0552	-0 04045 0 7672	0 59558 0 0001	1 00000 0 0000	0 62175 0 0001	0 23925 0 0757	0 21309 0 1148	0 32514 0 0145	-0 46362 0 0003	0 18151 0 1806
PRT12	0 35732 0 0069	0 51632 0 0001	0 65475 0 0001	0 63885 0 0001	0 02234 0 8702	0 64361 0 0001	0 62175 0 0001	1 00000 0 0000	0 16178 0 2336	0 11701 0 3903	0 75519 0 0001	-0 70815 0 0001	0 13520 0 3205
PRT20	0 00158 0 9908	0 01530 0 9109	0 06640 0 6268	0 07119 0 6021	0 62495 0 0001	0 04374 0 7489	0 23925 0 0757	0 16178 0 2336	1 00000 0 0000	0 16401 0 2271	0 20998 0 1201	-0 17085 0 2080	0 16384 0 2276
PIN	0 01114 0 9351	0 17157 0 2061	0 15462 0 2552	-0 17234 0 0002	-0 41413 0 0015	-0 09670 0 4783	0 21309 0 1148	0 11701 0 3903	0 16101 0 2271	1 00000 0 0000	0 04218 0 7559	0 12897 0 3435	0 10123 0 4578
NSOL	0 11911 0 0013	0 37937 0 0039	0 38868 0 0031	0 47915 0 0002	0 15273 0 2611	0 67083 0 0001	0 32514 0 0145	0 75519 0 0001	0 20998 0 1201	0 04248 0 7559	1 00000 0 0000	-0 71144 0 0001	0 24089 0 0737
DIE	-0 81860 0 0001	-0 50090 0 0001	0 41805 0 0013	-0 27924 0 0371	0 20685 0 1261	-0 52153 0 0001	-0 46362 0 0003	-0 70815 0 0001	0 17085 0 2080	-0 12897 0 3435	-0 71144 0 0001	1 00000 0 0000	-0 29311 0 0284
STEAM	0 21183 0 1118	-0 01990 0 8842	0 01739 0 8988	-0 33595 0 0114	-0 24461 0 0692	-0 04392 0 7479	0 18151 0 1806	0 13520 0 3205	0 16384 0 2276	0 10123 0 4578	0 24089 0 0737	-0 29311 0 0284	1 00000 0 0000
FDRATE	0 01259 0 9266	0 07456 0 5850	-0 05106 0 7086	-0 19182 0 1567	-0 26944 0 0446	-0 12526 0 3577	0 01752 0 8980	-0 25452 0 0584	-0 22996 0 0882	0 22015 0 1030	-0 38027 0 0038	0 15464 0 2551	0 01954 0 8863
TIME	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000
DM	0 01710 0 7303	0 16227 0 2321	0 18713 0 1679	0 15170 0 2644	0 06702 0 6236	0 17069 0 2085	0 14518 0 2847	0 13013 0 3380	0 02916 0 8311	0 03970 0 7714	0 09627 0 4803	-0 08206 0 5477	-0 03022 0 8250
PRT	0 09006 0 5092	0 16922 0 2135	0 18066 0 1827	0 10570 0 4382	0 05581 0 6827	0 18104 0 1818	0 18801 0 1653	0 17628 0 1937	0 10196 0 4111	0 02604 0 8489	0 14560 0 2813	0 13713 0 3135	0 02950 0 8291

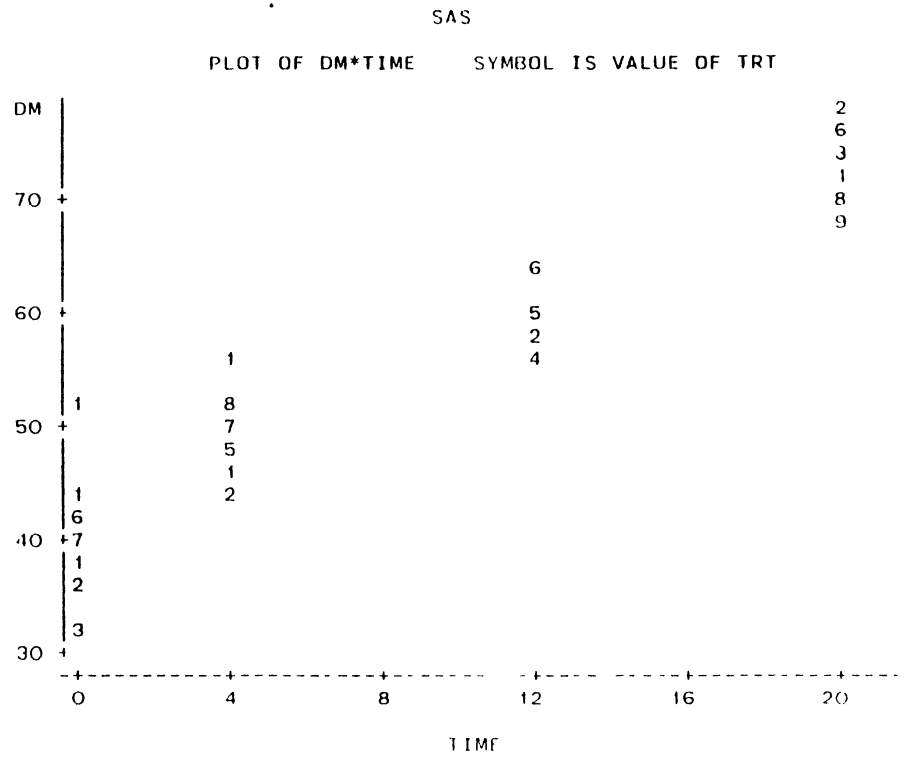
Correlation coefficients: Full-fat extruded soybeans

SAS

CORRELATION COEFFICIENTS / PROB > |R| UNDER HO RHO=0 / N = 56

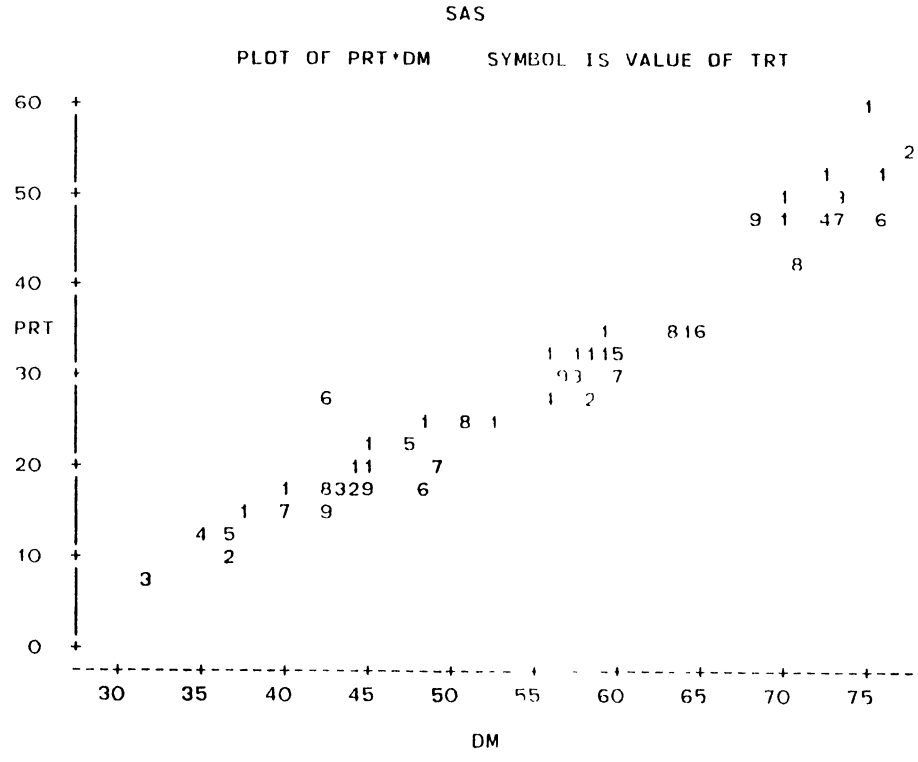
	FDRATE	TIME	DM	PRT
TRT	0 01259 0 9266	0 00000 1 0000	0 01710 0 7301	0 09006 0 5092
DMO	0 07456 0 5850	0 00000 1 0000	0 16227 0 2321	0 16922 0 2125
DM4	-0 05106 0 7086	0 00000 1 0000	0 18713 0 1673	0 18066 0 1827
DM12	0 19182 0 1567	0 00000 1 0000	0 15170 0 2641	0 10570 0 4382
DM20	0 26941 0 0446	0 00000 1 0000	0 06702 0 6236	0 05584 0 6827
PRTO	0 12526 0 3577	0 00000 1 0000	0 17069 0 2085	0 18101 0 1818
PRT4	0 01751 0 8980	0 00000 1 0000	0 11518 0 2817	0 18801 0 1653
PRT12	-0 25152 0 0584	0 00000 1 0000	0 13043 0 3380	0 17628 0 1937
PRT20	-0 22996 0 0882	0 00000 1 0000	0 02916 0 8311	0 10496 0 4414
PIN	0 22015 0 1030	0 00000 1 0000	0 03970 0 7714	0 02604 0 8489
NSOL	0 38017 0 0078	0 00000 1 0000	0 09627 0 4803	0 14560 0 2813
DIE	0 15461 0 2551	0 00000 1 0000	-0 08206 0 5477	0 13713 0 3135
STEAM	0 01951 0 8863	0 00000 1 0000	0 03022 0 8250	0 02950 0 8291
FDRATE	1 00000 0 0000	0 00000 1 0000	-0 01961 0 8858	-0 04074 0 7656
TIME	0 00000 1 0000	1 00000 0 0000	0 96074 0 0001	0 94080 0 0001
DM	-0 01964 0 8858	0 96074 0 0001	1 00000 0 0000	0 97163 0 0001
PRT	-0 04074 0 7656	0 94080 0 0001	0 97163 0 0001	1 00000 0 0000

Correlation coefficients: Full-fat extruded soybeans



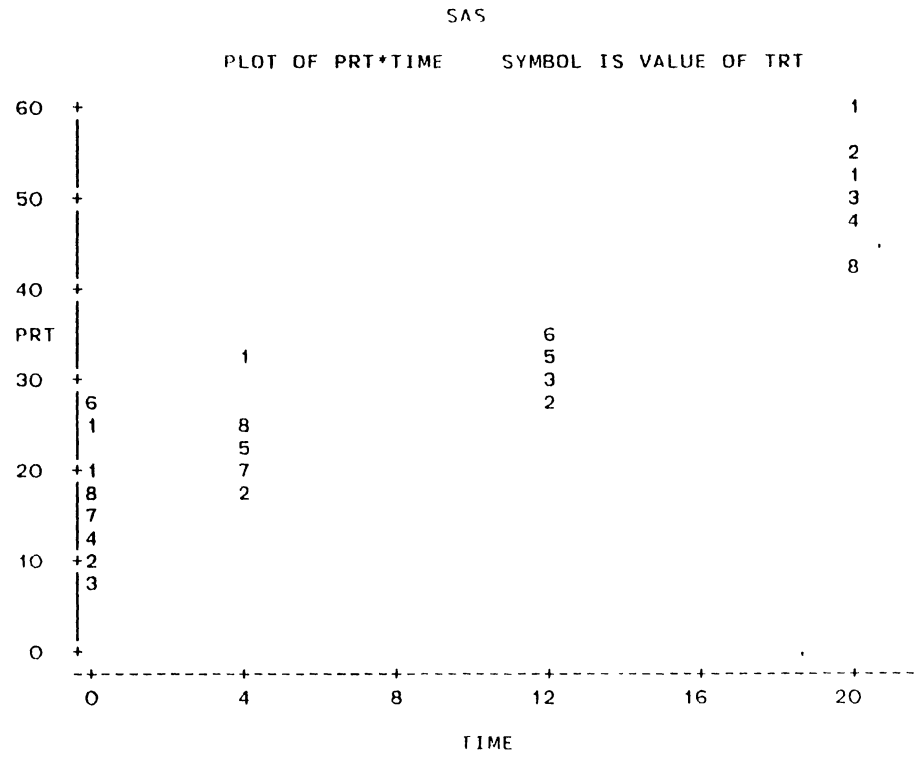
NOTE 33 OBS HIDDEN

Plot of disappearance values for full-fat extruded soybeans from dacron bags in rumen of a cow



NOTE 8 OBS HIDDEN

Plot of disappearance values for full-fat extruded soybeans from dacron bags in rumen in a cow



NOTE 33 OBS HIDDEN

Plot of disappearance values for full-fat extruded soybeans from dacron bags in rumen in a cow

CAS

CORRELATION COEFFICIENTS / PROB > |R| UNDER HO RHO=0 / N = 60

	TRT	DMO	DM4	DM12	DM20	PRT0	PRT4	PRT12	PRT20	PIN	NSOL	WATER	STEAM
TRT	1 00000 0 0000	0 34802 0 0064	0 19515 0 1351	-0 32000 0 0127	-0 48419 0 0001	0 01053 0 9364	0 23061 0 0763	0 19609 0 1332	-0 31520 0 0142	-0 26071 0 0442	0 14314 0 2752	0 08452 0 5209	0 12677 0 3344
DMO	0 34802 0 0064	1 00000 0 0000	0 08093 0 5388	-0 28929 0 0250	-0 33458 0 0090	0 40904 0 0012	-0 16388 0 2109	0 24704 0 0571	-0 43920 0 0004	-0 21959 0 0918	0 01352 0 9184	-0 49795 0 0001	-0 07833 0 5519
DM4	0 19515 0 1351	0 08093 0 5388	1 00000 0 0000	0 40744 0 0012	0 21788 0 0944	0 45796 0 0002	0 84942 0 0001	-0 02503 0 8494	-0 02738 0 8355	-0 03255 0 8050	0 25962 0 0452	-0 32782 0 0106	-0 19004 0 1458
DM12	-0 32000 0 0127	-0 28929 0 0250	0 40744 0 0012	1 00000 0 0000	-0 09110 0 4888	0 09344 0 4776	0 36073 0 0046	0 55929 0 0001	-0 30122 0 0193	0 11637 0 3759	0 32966 0 0101	-0 02836 0 8297	-0 25289 0 0512
DM20	-0 48419 0 0001	-0 33458 0 0090	0 21788 0 0944	-0 09110 0 4888	1 00000 0 0000	0 09046 0 4919	0 23925 0 0656	-0 24729 0 0568	0 57927 0 0001	0 39617 0 0017	-0 34643 0 0067	0 06450 0 6244	0 39458 0 0018
PRT0	0 01053 0 9364	0 40904 0 0012	0 45796 0 0002	0 09344 0 4776	0 09046 0 1919	1 00000 0 0000	0 54459 0 0001	0 23169 0 0749	0 04089 0 7564	-0 61485 0 0001	0 47331 0 0001	-0 37362 0 0033	0 31501 0 0142
PRT4	-0 23061 0 0763	0 16388 0 2109	0 84942 0 0001	0 36073 0 0046	0 23925 0 0656	0 54459 0 0001	1 00000 0 0000	-0 05214 0 6923	0 25779 0 0467	-0 19284 0 1399	0 42512 0 0007	-0 27181 0 0357	-0 01583 0 9044
PRT12	-0 19609 0 1332	0 24704 0 0571	-0 02503 0 8494	0 55929 0 0001	-0 24729 0 0568	0 23169 0 0749	-0 05214 0 6923	1 00000 0 0000	-0 51342 0 0001	0 06971 0 5966	0 38741 0 0022	-0 39912 0 0016	-0 09922 0 4507
PRT20	-0 31520 0 0142	-0 43920 0 0004	-0 02738 0 8355	-0 30122 0 0193	0 57927 0 0001	0 04089 0 7564	0 25779 0 0467	-0 51342 0 0001	1 00000 0 0000	-0 08508 0 5181	-0 24078 0 0639	0 35769 0 0050	0 49122 0 0001
PIN	-0 26071 0 0442	-0 21959 0 0918	-0 03255 0 8050	0 11637 0 3759	0 39617 0 0017	-0 61485 0 0001	-0 19284 0 1399	0 06971 0 5966	-0 08508 0 5181	1 00000 0 0000	-0 47217 0 0001	0 09762 0 4581	-0 23428 0 0716
NSOL	-0 14314 0 2752	0 01352 0 9184	0 25962 0 0452	0 32966 0 0101	-0 34643 0 0067	0 47331 0 0001	0 42512 0 0007	0 38741 0 0022	-0 24078 0 0639	-0 47217 0 0001	1 00000 0 0000	-0 27397 0 0342	-0 13699 0 2966
WATER	0 08452 0 5209	-0 49795 0 0001	-0 32782 0 0106	-0 02836 0 8297	0 06450 0 6244	-0 37362 0 0033	-0 27181 0 0357	-0 39942 0 0016	0 35769 0 0050	0 09762 0 4581	-0 27397 0 0342	1 00000 0 0000	0 00000 1 0000
STEAM	0 12677 0 3344	-0 07833 0 5519	-0 19004 0 1458	-0 25289 0 0512	0 39458 0 0018	0 31501 0 0142	-0 01583 0 9044	-0 09922 0 4507	0 49122 0 0001	-0 23428 0 0716	-0 13699 0 2966	0 00000 1 0000	1 00000 0 0000
FDRATE	0 16903 0 1967	-0 00559 0 9662	0 25181 0 0523	0 57906 0 0001	-0 50461 0 0001	0 30769 0 0168	0 28236 0 0288	0 31547 0 0141	-0 35292 0 0057	-0 39047 0 0020	0 64758 0 0001	0 00000 1 0000	0 00000 1 0000
TEMP	-0 14814 0 2587	-0 26269 0 0426	-0 15645 0 2326	0 15459 0 2383	-0 48945 0 0001	-0 10239 0 4363	0 06112 0 6427	0 09195 0 4847	-0 06623 0 6151	-0 29231 0 0234	0 44783 0 0003	-0 22617 0 0823	-0 02827 0 8302
TIME	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000	0 00000 1 0000
DM	-0 03656 0 7816	0 03335 0 8003	0 09546 0 4681	0 07760 0 5556	0 03078 0 8154	0 06225 0 6365	0 06834 0 6039	0 05178 0 6944	-0 02798 0 8319	0 01390 0 9161	0 02190 0 8681	-0 04924 0 7087	-0 01445 0 9128

Correlation coefficients: 44% solvent extracted soybeans

SAS

CORRELATION COEFFICIENTS / PROB > |R| UNDER HO RHO=0 / N = 60

	TRT	DMO	DM4	DM12	DM20	PRTO	PRT4	PRT12	PRT20	PIN	NSOL	WATER	STEAM
PRT	0 07210 0 5825	0 00907 0 9452	0 10288 0 4341	0 06182 0 6389	0 06311 0 6319	0 14204 0 2790	0 11872 0 2568	0 05348 0 6819	0 07890 0 5490	0 06021 0 6175	0 08258 0 5305	0 05125 0 6974	0 05971 0 6504

	FDRATE	TEMP	TIME	DM	PRT
TRT	0 16901 0 1967	0 11811 0 2587	0 00000 1 0000	0 03656 0 7816	-0 07210 0 5825
DMO	-0 00559 0 9662	-0 26269 0 0426	0 00000 1 0000	0 03335 0 8003	-0 00907 0 9452
DM4	0 25181 0 0523	0 15645 0 2326	0 00000 1 0000	0 09546 0 4681	0 10288 0 4341
DM12	0 57906 0 0001	0 15459 0 2383	0 00000 1 0000	0 07760 0 5556	0 06182 0 6389
DM20	-0 50161 0 0001	-0 18915 0 0001	0 00000 1 0000	0 03078 0 8154	0 06311 0 6319
PRTO	0 30769 0 0168	0 10233 0 4363	0 00000 1 0000	0 06225 0 6365	0 14204 0 2790
PRT4	0 28236 0 0288	0 06112 0 6427	0 00000 1 0000	0 06834 0 6039	0 11872 0 2568
PRT12	0 31517 0 0141	0 09195 0 4847	0 00000 1 0000	0 05178 0 6941	0 05348 0 6849
PRT20	-0 35292 0 0057	-0 06623 0 6151	0 00000 1 0000	-0 02798 0 8319	0 07890 0 5490
PIN	-0 39047 0 0020	-0 29231 0 0234	0 00000 1 0000	0 01390 0 9161	-0 06024 0 6475
NSOL	0 61758 0 0001	0 14783 0 0003	0 00000 1 0000	0 02190 0 8681	0 08258 0 5305
WATER	0 00000 1 0000	-0 22617 0 0823	0 00000 1 0000	-0 04924 0 7087	-0 05125 0 6974
STEAM	0 00000 1 0000	0 02827 0 8302	0 00000 1 0000	-0 01445 0 9128	0 05971 0 6504
FDRATE	1 00000 0 0000	0 36752 0 0039	0 00000 1 0000	0 03316 0 8014	0 04037 0 7594
TEMP	0 36752 0 0039	1 00000 0 0000	0 00000 1 0000	-0 03874 0 7688	0 00086 0 9948
TIME	0 00000 1 0000	0 00000 1 0000	1 00000 0 0000	0 96446 0 0001	0 93103 0 0001

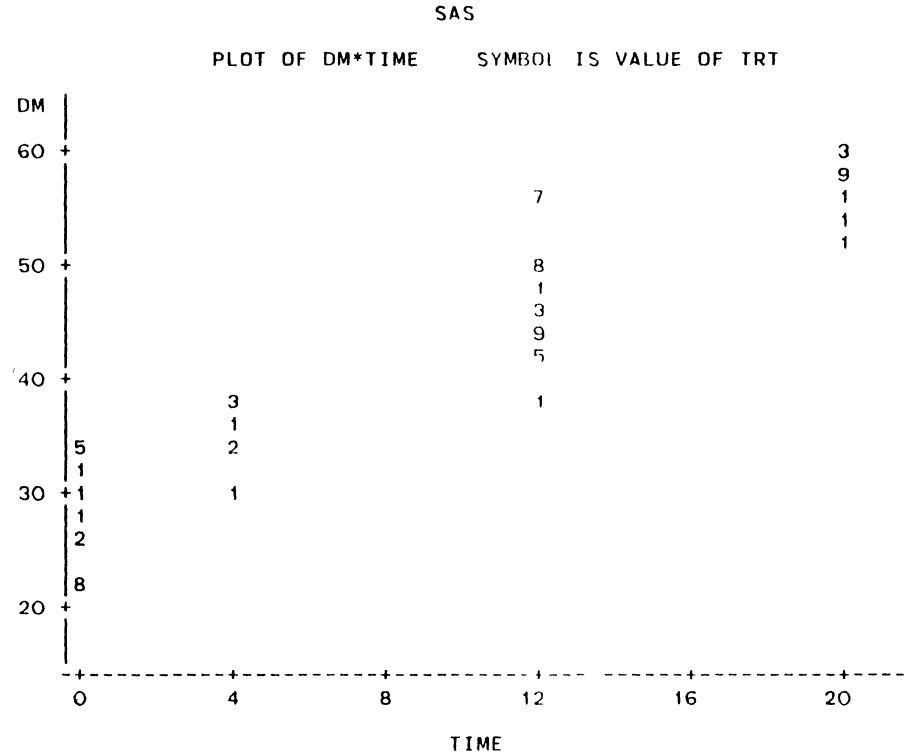
Correlation coefficients: 44% solvent extracted soybeans

SAS

CORRELATION COEFFICIENTS / PROB > |R| UNDER HO RHO=0 / N = 60

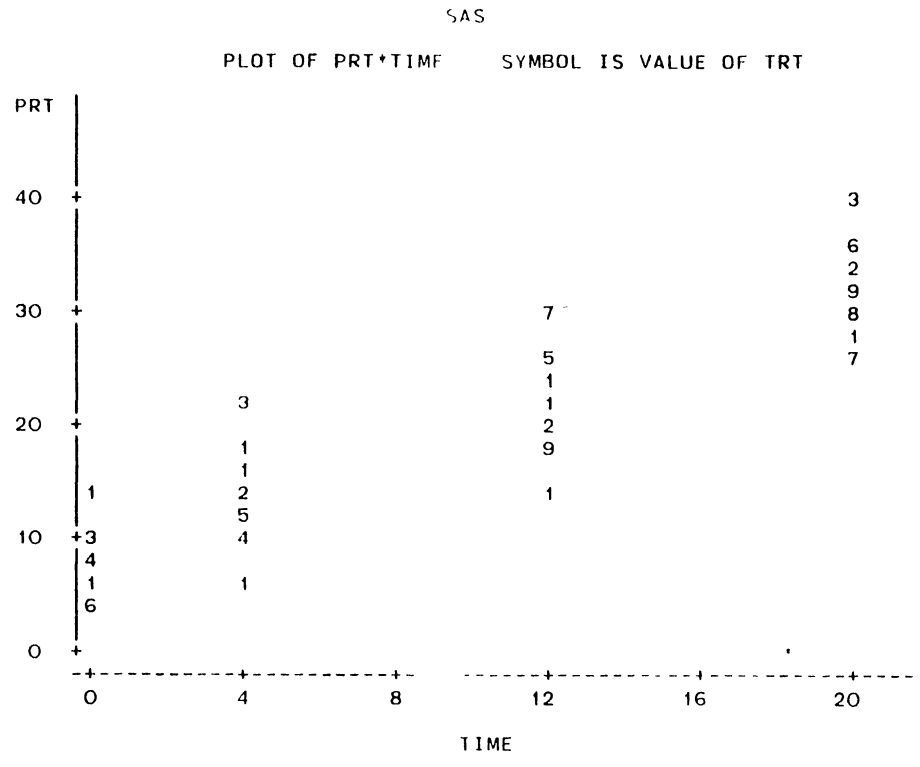
	FDRATE	TEMP	TIME	DM	PRT
DM	0 03316 0 8014	-0 03874 0 7688	0 96446 0 0001	1 00000 0 0000	0 95022 0 0001
PRT	0 04037 0 7594	0 00086 0 9948	0 93103 0 0001	0 95022 0 0001	1 00000 0 0000

Correlation coefficients: 44% solvent extracted soybeans



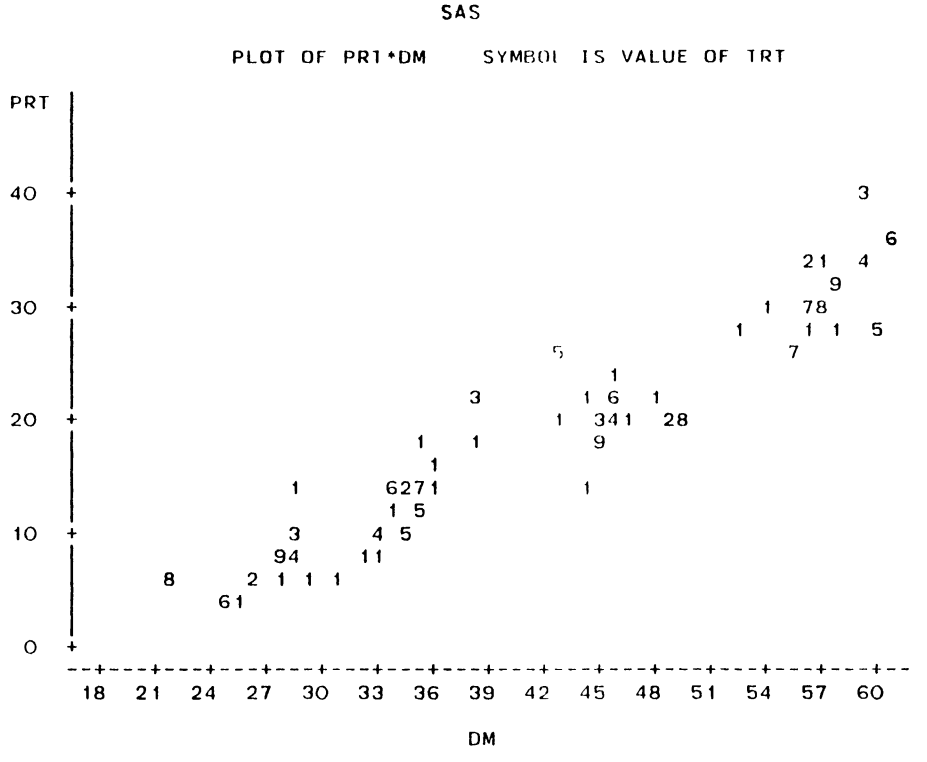
NOTE 38 OBS HIDDEN

Plot of disappearance values for 44% solvent extracted soybeans from dacron bags in rumen of a cow



NOTE 34 OBS HIDDEN

Plot of disappearance values for 44% solvent extracted soybeans from dacron bags in rumen of a cow



NOTE 8 OBS HIDDEN

Plot of disappearance values for 44% solvent extracted soybeans from dacron bags in rumen of a cow

APPENDIX B

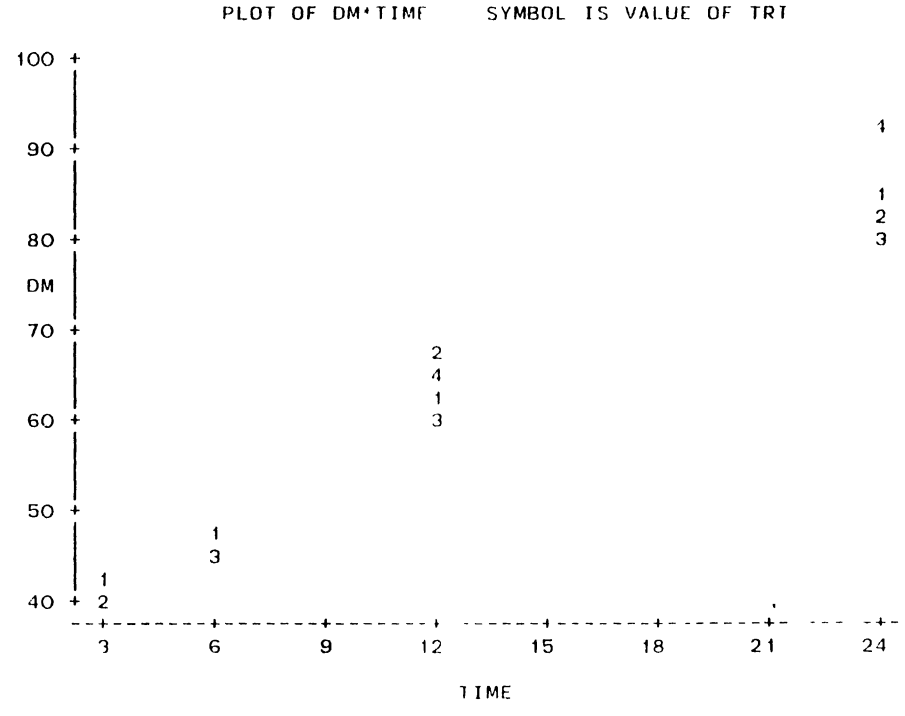
DRY MATTER DISAPPEARANCE AND NITROGEN DISAPPEARANCE PLOTS OF FEED INGREDIENTS STUDIED IN CHAPTER VII

TRT 1 = 65% conc diet with SBM

TRT 2 = 65% conc diet with urea

TRT 3 = 35% conc diet with SBM

TRT 4 = 35% conc diet with urea



NOTE 4 OBS HIDDEN

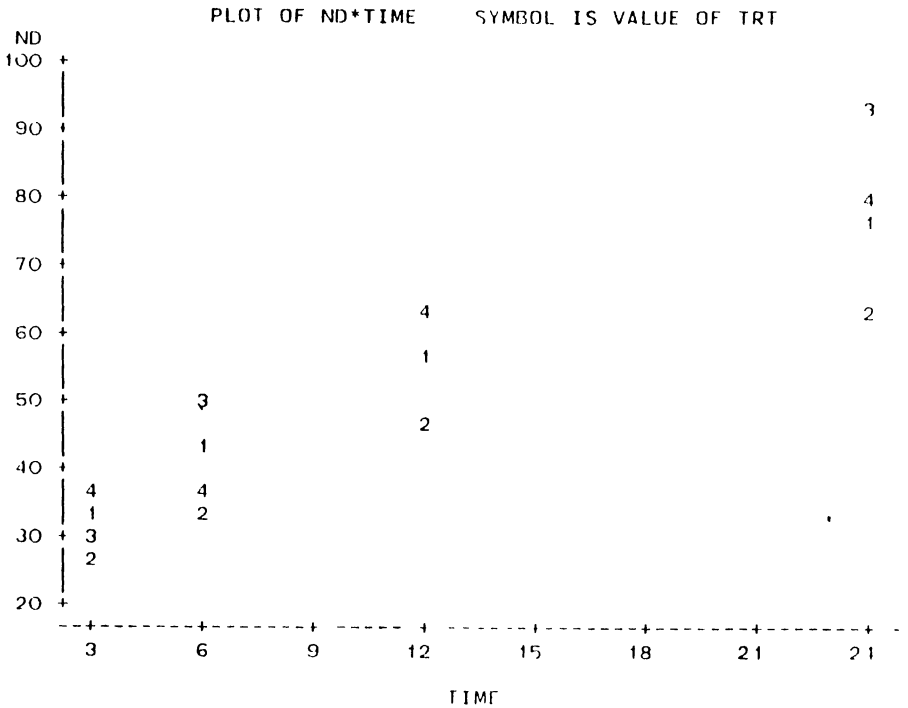
Plot of disappearance values for soybean meal from dacron bags in rumen of cow fed experimental diets

TRT 1 = 65% conc diet with SBM

TRT 2 = 65% conc diet with urea

TRT 3 = 35% conc diet with SBM

TRT 4 = 35% conc diet with urea



NOTE 1 OBS HIDDEN

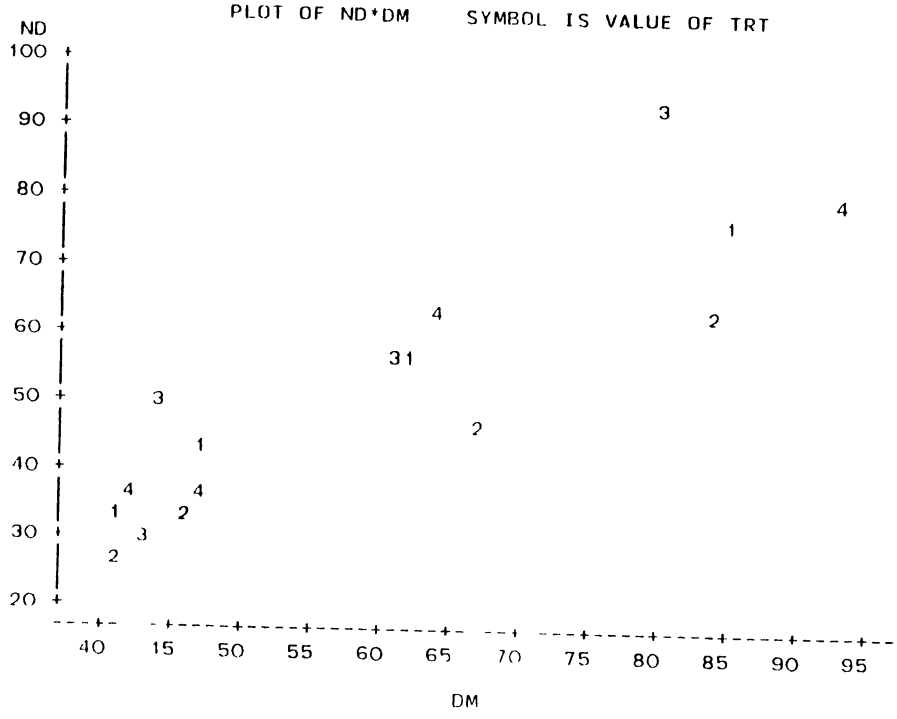
Plot of disappearance values for soybean meal from dacron bags in rumen of cow fed experimental diets

TRT 1 = 65% conc diet with SBM

TRT 2 = 65% conc diet with urea

TRT 3 = 35% conc diet with SBM

TRT 4 = 35% conc diet with urea



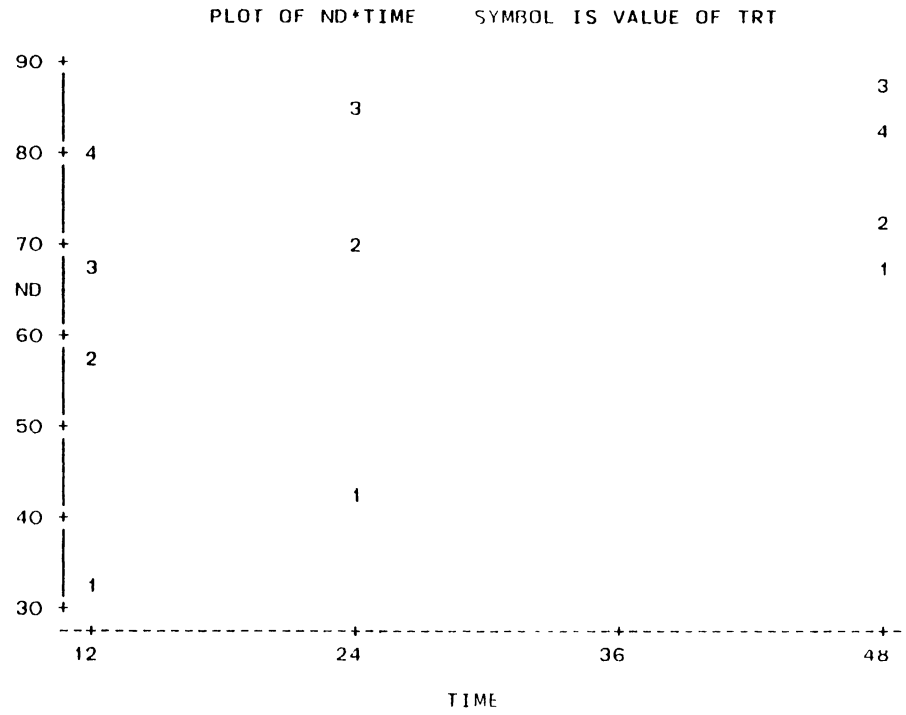
Plot of disappearance values for soybean meal from dacron bags in rumen of cow fed experimental diets

TRT 1 = 65% conc diet with SBM

TRT 2 = 65% conc diet with urea

TRT 3 = 35% conc diet with SBM

TRT 4 = 35% conc diet with urea



NOTE 1 OBS HIDDEN

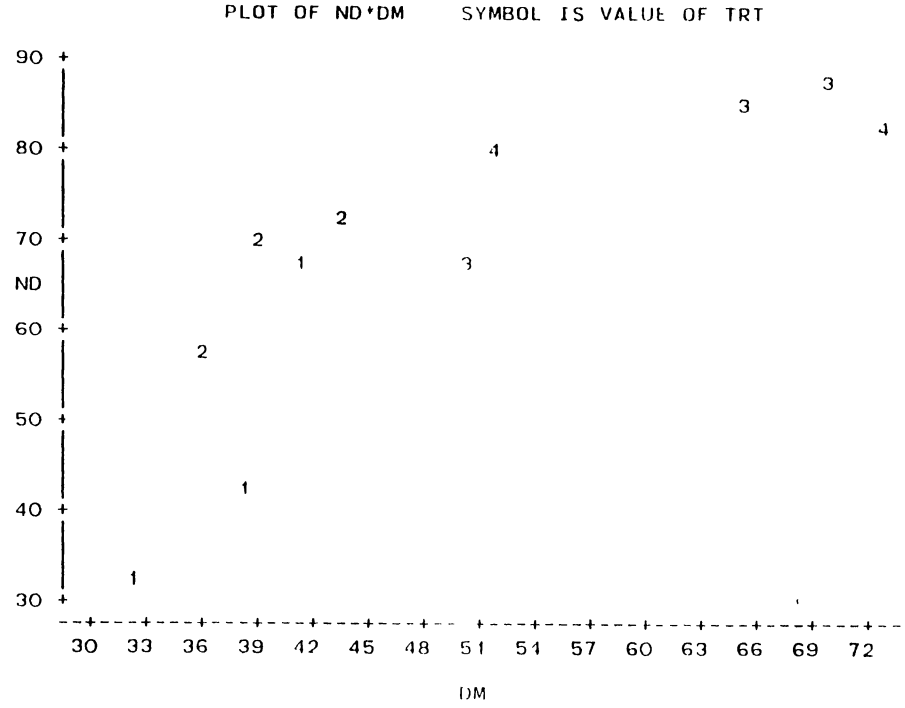
Plot of disappearance values for sorghum silage from dacron bags in rumen of cow fed experimental diets

TRT 1 = 65% conc diet with SBM

TRT 2 = 65% conc diet with urea

TRT 3 = 35% conc diet with SBM

TRT 4 = 35% conc diet with urea



NOTE 1 OBS HIDDEN

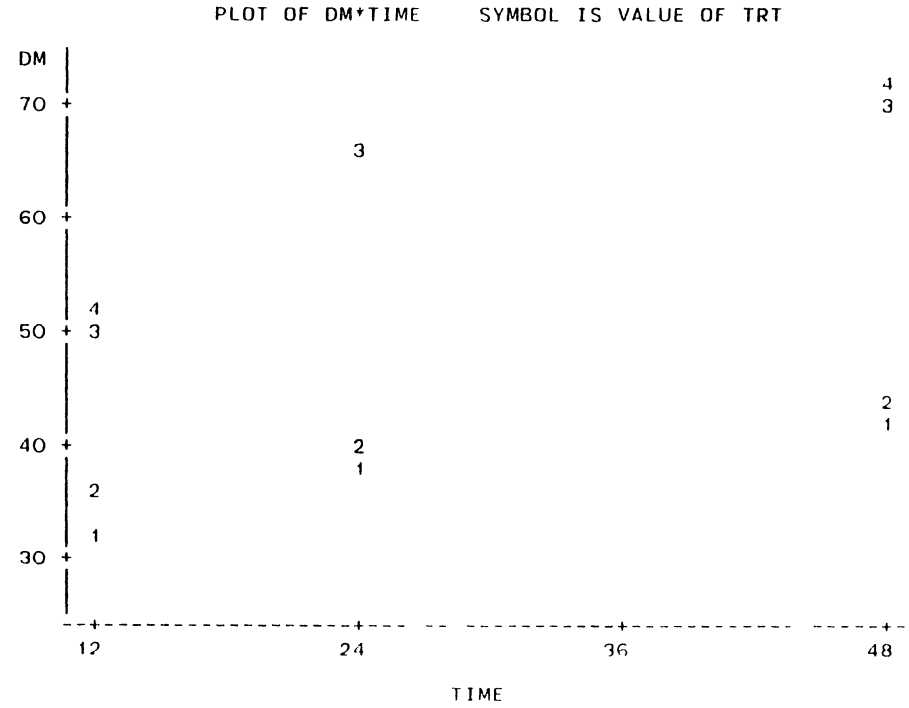
Plot of disappearance values for sorghum silage from dacron bags in rumen of cow fed experimental diets

TRT 1 = 65% conc diet with SBM

TRT 2 = 65% conc diet with urea

TRT 3 = 35% conc diet with SBM

TRT 4 = 35% conc diet with urea



NOTE 1 OBS HIDDEN

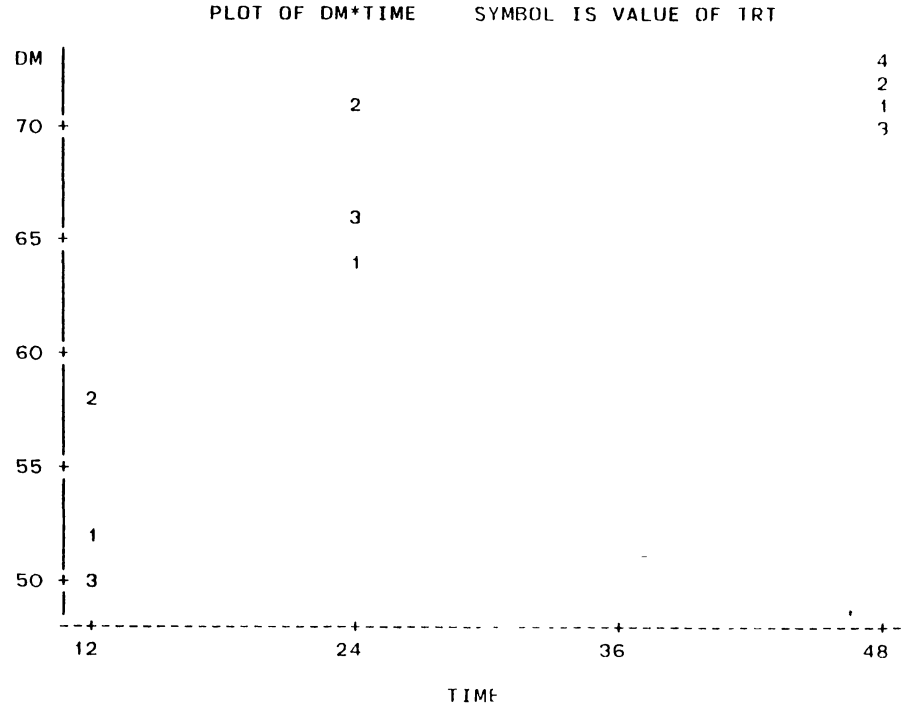
Plot of disappearance values for sorghum silage from dacron bags in rumen of cow fed experimental diets

TRT 1 = 65% conc diet with SBM

TRT 2 = 65% conc diet with urea

TRT 3 = 35% conc diet with SBM

TRT 4 = 35% conc diet with urea



NOTE 2 OBS HIDDEN

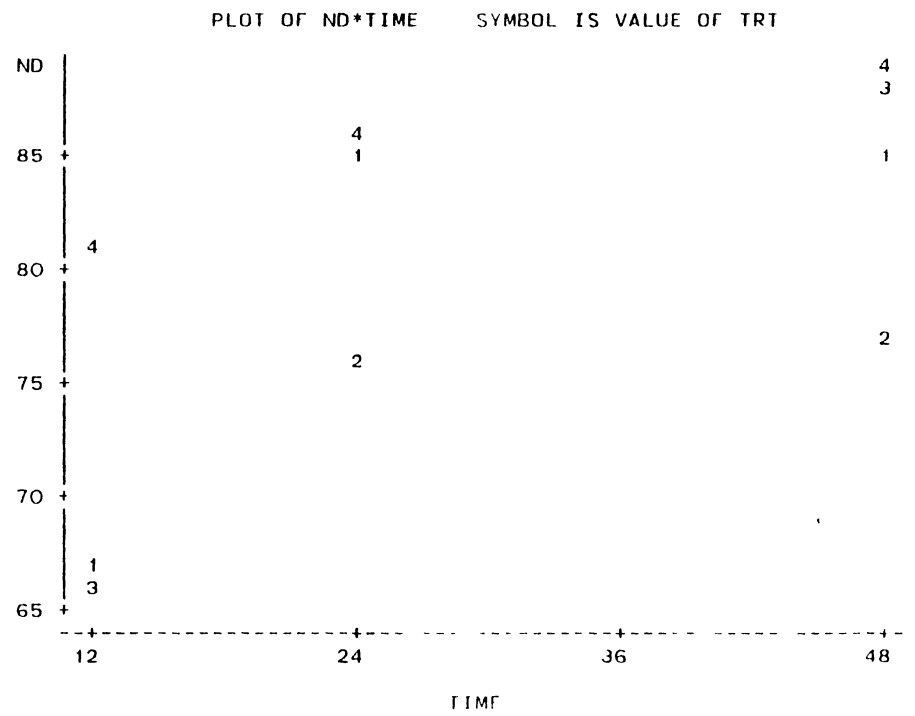
Plot of disappearance values for alfalfa hay from dacron bags in rumen of cow fed experimental diets

TRT 1 = 65% conc diet with SBM

TRT 2 = 65% conc diet with urea

TRT 3 = 35% conc diet with SBM

TRT 4 = 35% conc diet with urea



NOTE 2 OBS HIDDEN

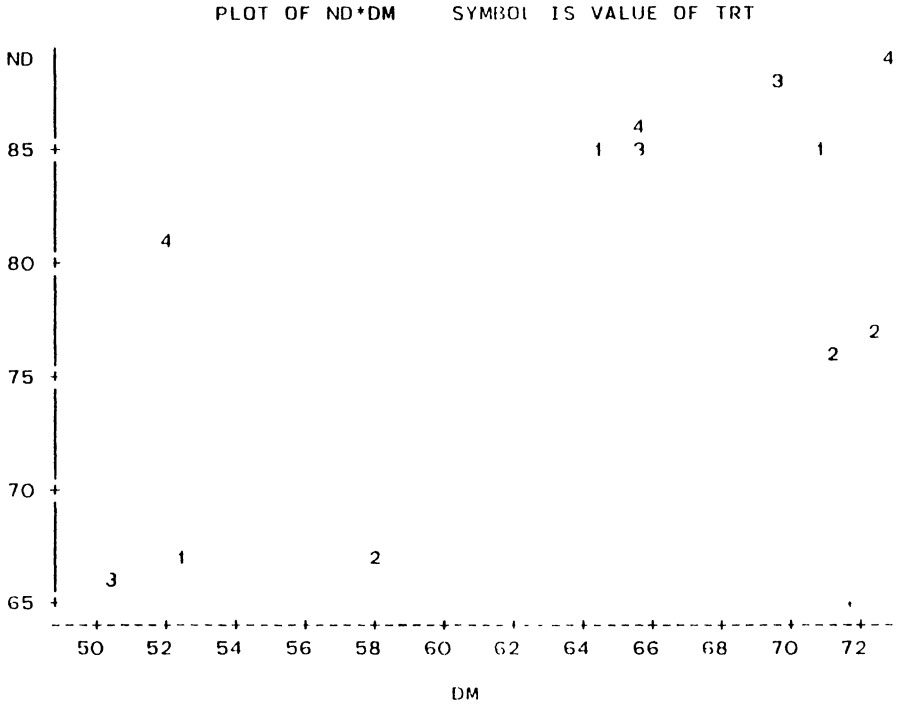
Plot of disappearance values for alfalfa hay from dacron bags in rumen of cow fed experimental diets

TRT 1 = 65% conc diet with SBM

TRT 2 = 65% conc diet with urea

TRT 3 = 35% conc diet with SBM

TRT 4 = 35% conc diet with urea



Plot of disappearance values for alfalfa hay from dacron bags in rumen of cow fed experimental diets

VITA

Joseph William Ward

Candidate for the Degree of

Doctor of Philosophy

Thesis: FACTORS AFFECTING THE UTILIZATION OF PROTEIN IN SOYBEAN MEAL BY DAIRY COWS

Major Field: Animal Nutrition

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Experience: Raised on a farm in northern Indiana. Veterinary Assistant, New Carlisle Animal Clinic, 1968-1971; Youth Extension Trainee, La Porte County, IN, Summer of 1972; Undergraduate Research Assistant, Purdue University, 1974-1975; Research Graduate Assistant, Purdue University, 1975-1977; Research and Development Coordinator, Agrimerica, Inc., Northbrook, IL, 1978-1979; Graduate Assistant, Oklahoma State University, 1978-1982; Vice-President, Director of Research and Technical Services, Agrimerica, Inc., Northbrook, IL, 1982-1984.

Professional Organizations: American Society of Animal Science, American Dairy Science Association, American Association of Cereal Chemists, Council of Agricultural Science and Technology, CERES and Alpha Zeta.